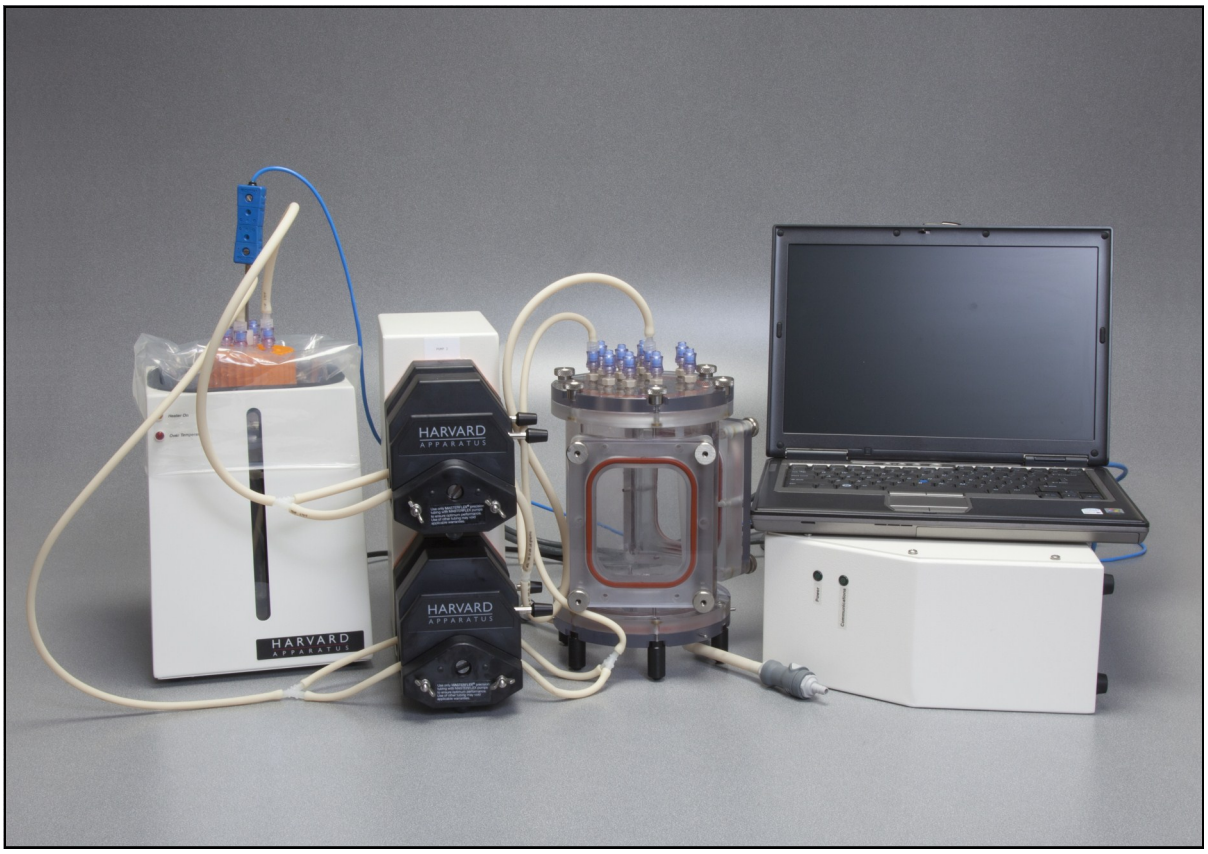
ORCA Bioreactor™

Operator's Manual



HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

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Disclaimer:

Use of the ORCA Bioreactor™ should be conducted by a trained and manufacturer qualified representative. Harvard Apparatus Regenerative Technology does not warrant unauthorized use of this product; Harvard Apparatus does not warrant that the operation of this product will be uninterrupted or error-free and makes no claim of warranty or condition.

HART reserves the right to change the instructions for use and any related products at any time without any prior notice and is not liable for any damages arising out of any change and/or alteration of the contents or product.

This product is for RESEARCH USE ONLY.

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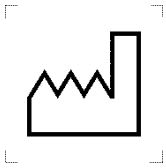
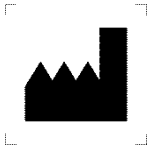
U.S., international, and foreign patent applications are pending.

Symbols Used

Symbol	Definition
	Date of Manufacture
	Serial Number
	Catalogue Number
	Warning: This action will have a direct impact on the patient
	Caution: This action will have an impact on the product or operator
	Manufacturer
CE	This device complies with Directive 2006/95/EC relating to electrical equipment designed for use within certain voltage limits; this device also complies with Directive 2004/108/EC relating to electromagnetic compatibility.

Overview

HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

Harvard Apparatus Regenerative Technology (HART) partners with leading global scientists to provide specialized solutions.

The company is uniquely positioned to develop advanced instrumentation to accelerate regenerative medicine, tissue engineering and cell therapy experimentation. From the beginning, we worked closely with leading global researchers to produce products with the highest levels of performance, quality and support necessary for the new challenges of your life science research.

We look forward to working with you to develop new tools to assist you in solving the new challenges of regenerative medicine from the lab bench to the patient. There are thousands of publications, in regenerative medicine to stem cell research, utilizing HART products, but we are now introducing some newly developed products: one for regenerative organ generation and one for small volume cell delivery into organs. These products will serve the researcher and the physicians to accelerate the research and utilization of that research in patients.

Chapter 1: Introduction

The **ORCA Bioreactor** is the first system designed to meet the needs of the modern regenerative medicine research scientist. The system is able to address the needs of both the decellularization and recellularization processes of various organs.

Physiological conditions can be mimicked through control algorithms that regulate flow rates, profiles, and pressures. Critical readings can be taken from both inside the organ as well as in the support environment through the use of sensors.

The Concurrent Method Development software logs all modifications and operator entries along with the result that was measured with the system sensors, allowing a complete review of an experiment and direct translation into a research method.

A method storage system makes it easy to reproduce methods and conditions across multiple experiments.

The image capture software allows for images to be captured in real time from cameras monitoring visible light, IR, UV, and fluorescence; other systems such as ultrasound / ECHO are supported.

Chapter 2: Getting Started

2.1 Warning and Caution Statements

The use of a **WARNING** statement in this User Manual alerts you to a potential safety hazard. Failure to observe a warning may result in a serious injury to the user.

The use of a **CAUTION** statement in this User Manual alerts you to where special care is necessary for the safe and effective use of the product. Failure to observe a caution may result in minor injury to the user or damage to the product or other property.

2.2 Intended Use

The ORCA Bioreactor is a system used for the purposes of monitoring and studying an isolated organ; it is intended for research use only.

WARNING:

Use of this device in non-research settings must be conducted under local Regulatory requirements; consult your local Regulatory Authority.

2.3 General Safety Requirements

The following conditions must be met prior to using the ORCA Bioreactor:

WARNING:

The ORCA Bioreactor should only be used by qualified personnel who have been trained by the manufacturer or other Authorized Representative. Unauthorized use of this device is not recommended.

WARNING:

To prevent contamination, aseptic procedures must be followed and personal protective equipment must be worn at all times when handling and using the Bioreactor.

WARNING:

Wherever blood products are used, Universal Precautions must be followed.

Facility Requirements

Assure that the facility is able to provide a clean, safe, and suitable area for aseptic cell processing. It is recommend that all manipulations of the unit once sterile are performed in a biological safety cabinet (laminar flow hood).

WARNING:

Failure to provide a means to conduct aseptic cell processing may result in harmful contamination.

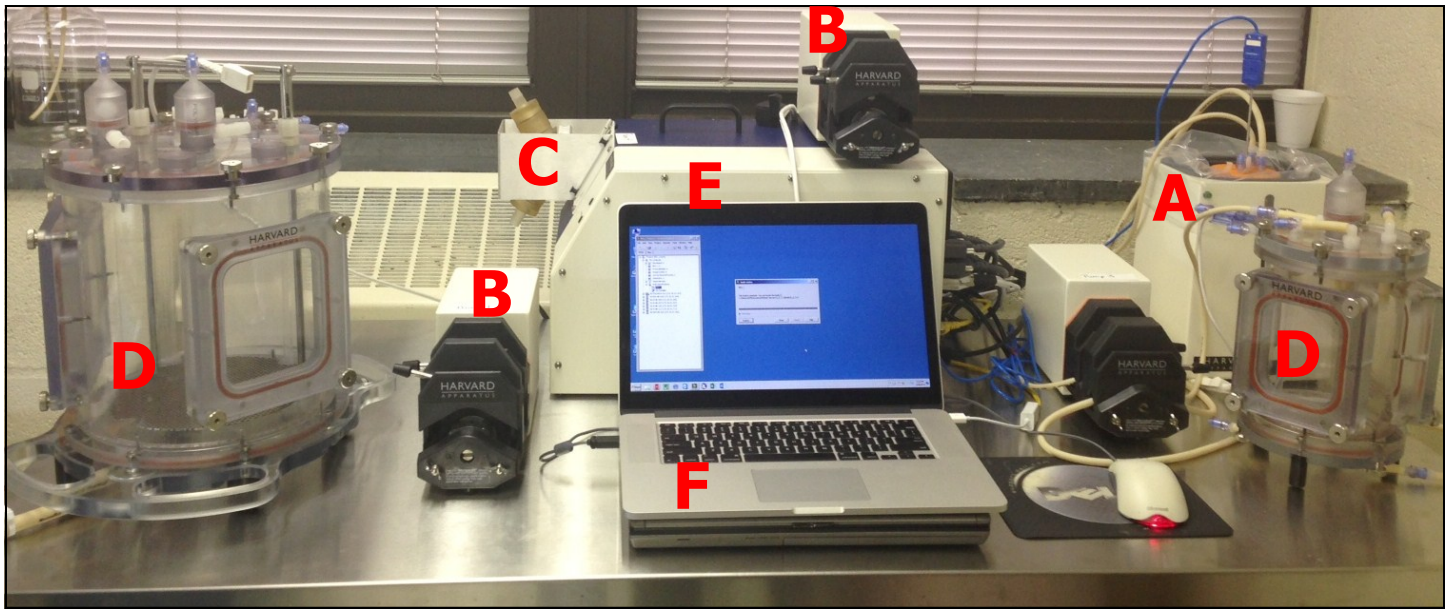
2.4 Equipment Components

It is important to:

1. Ensure that the ORCA Bioreactor package was received completely and without damage; if the package arrived as damaged contact your local technical support group. Do not use damaged parts.
2. Store the ORCA Bioreactor in a cool, dry place, free from dust and other potential contaminants until ready to use.

Overview of Main Components

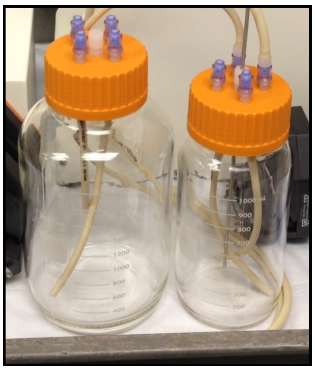
(A) Heater Reservoir
(B) Peristaltic Pumps
(C) Pulsatile Pump
(D) Organ Chambers
(E) ORCA Controller
(F) Laptop with Data Acquisition Software



Heater Reservoir

The heater is used to maintain temperature throughout the system to **emulate physiological conditions**.

Reservoirs have **built-in ports** for interaction with the organ chamber and the oxygenation system, and a special port for the thermocouple which monitors temperature within the reservoir.



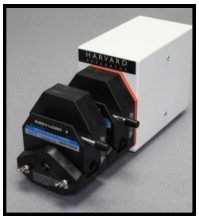
Pumps

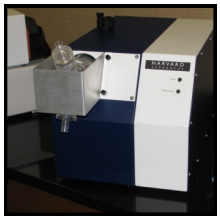
Pumps are selected according to the organ and protocol requirements. The ORCA controller along with your pumps of choice address the needs of the **decellularization** and **recellularization** processes of various organs. Several types of pumps may be controlled, including peristaltic and pulsatile.

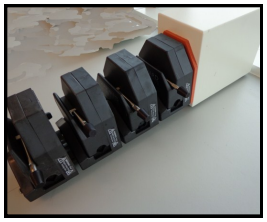
A maximum of four pumps are used simultaneously on a system. The ORCA controller may be configured to control either four peristaltic pumps or three peristaltic pumps and a pulsatile blood pump. Pump head configurations may be altered at any time to meet the most demanding protocols.

Selection of the pulsatile pump normally occurs when pulsatile flow rates in excess of 2L/min are required. This selection must be made at the time of purchase. If the "four peristaltic pump controller" is chosen, pumps may be added at any time. Additional peristaltic pumps add flexibility to accommodate elaborate protocols but are not required.

These configurations can be used to accommodate protocols that require a perfusion pump, ventilation pump and a pump that will provide flow to the oxygenation system and a gas monitoring system. Exterior medium feed and waste bags can also be accessed using channels on the double head double channel pump.







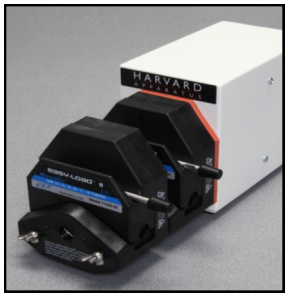
Pumps

Peristaltic Pumps

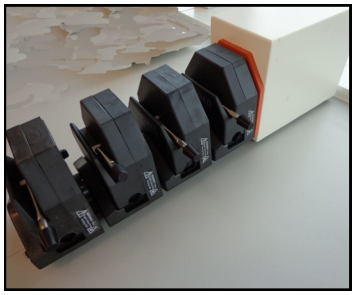
Peristaltic pump heads are available in **single** and **dual channel** versions. (High Capacity: up to 1200mL/min/head; Mid Capacity: up to 500mL/min/head)

Up to four (4) heads may be used on each peristaltic pump to provide flexibility in flow range as well as the number of channels required for the most challenging protocols.

One Single-channel Head {31061}



Two Single-channel Heads {31062}



Four Single-channel Heads {31063}

One Dual-channel Head {31064}

Two Dual-channel Heads {31065}

One each Single- and Dual-channel Heads {31066}

Additional Part Numbers:

{31067} Mounting Screw for Two pump heads
{31068} Mounting Screw for Three pump heads
{31069} Mounting Screw for Four pump heads

Pulsatile Pumps

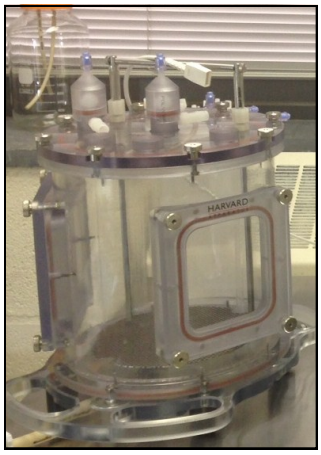
The pulsatile pump is used in some large animal systems to emulate ventricular action of the heart.

It allows for minimal hemolysis and is ideal for moving emulsions, suspensions, and blood.

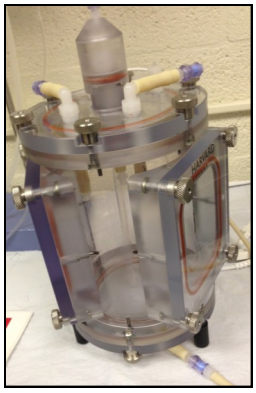
Chambers

Solid Organ Chambers

Large Solid Organ Chamber 14” {30240}
(holds approx. 32.8L)



Intermediate Solid Organ Chamber 10” {30260}
(holds approx. 11.6L)



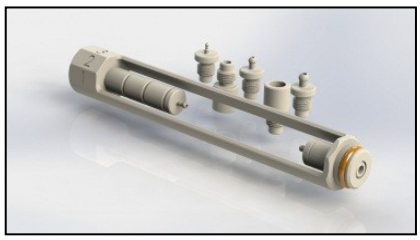
Small Solid Organ Chamber 5.5” {30320}
(holds approx. 2L)

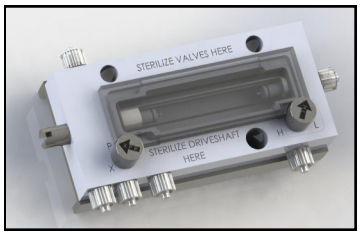
Solid organ chambers are autoclavable and are suitable for multiple species and sizes of organs. Tubing sets can be changed to accommodate the wide range of flow rates.

The layout of the chamber and use of clear materials allows for easy visibility of the organ being studied. Chamber orientation can be adjusted depending on the decellularization and recellularization procedures.

Chamber access ports are built in for removal and addition of media. Numerous sampling ports are included, and the built-in windows allow for manual access to an organ.

Hollow Organ Chambers





{30300}

The rotating double chamber is specifically designed for cell seeding and culturing both surfaces of a tubular matrix. The **intraluminal** and **extraluminal** flow paths may be connected or maintained separately. The hollow organ chambers allow seeding and culturing of different cells types on either side of the tubular structure, providing homogeneity. The design allows for enhanced oxygenation and mass transport between the medium and cells.

Large and small animal versions are available. See the Hollow Organ Bioreactor manual for additional information.

Chapter 3: Assembly Instructions

3.1 Identifying Components

This is a typical system. Actual components will vary according to the system ordered. Please check your order and shipping manifest for an exact list of components.

Pumps

Pump Cables {31072}

Pump Drive with appropriate heads (1 to 4). The type and number of heads will vary with each system.

Pressure Transducers

4 channel controller cable {31073}

Transducers {31074} are shipped in sealed sterile packaging

Laptop {31082}

Laptop and power cables will vary depending on the order

Manual Pressure Calibrator {31075}

Controller

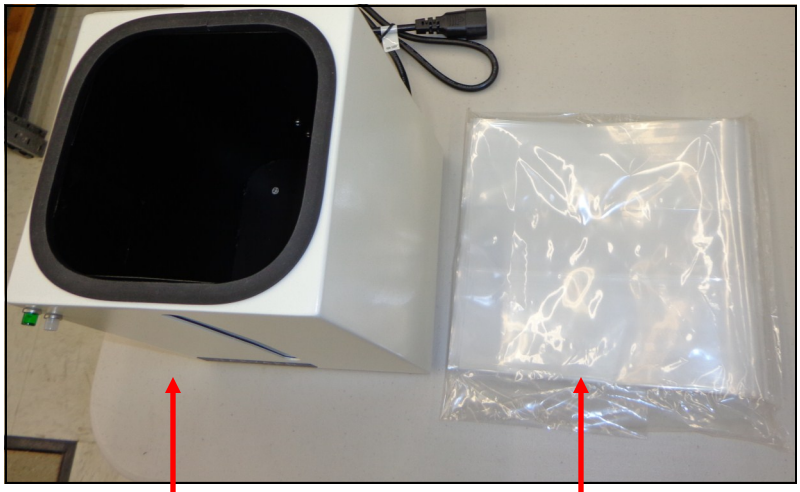
Communications cable {32077}
DO NOT PLUG THE COMMUNICATIONS CABLE INTO A ETHERNET PLUG AND CONNECT IT TO THE NETWORK. THE LAPTOP COULD CHANGE THE IP ADDRESS AND NOT BE ABLE TO COMMUNICATE. YOU WILL THEN HAVE TO MANUALLY CHANGE THE IP ADDRESS BACK TO 172.16.51.59 (the subnet mask should be 255.255.255.0).

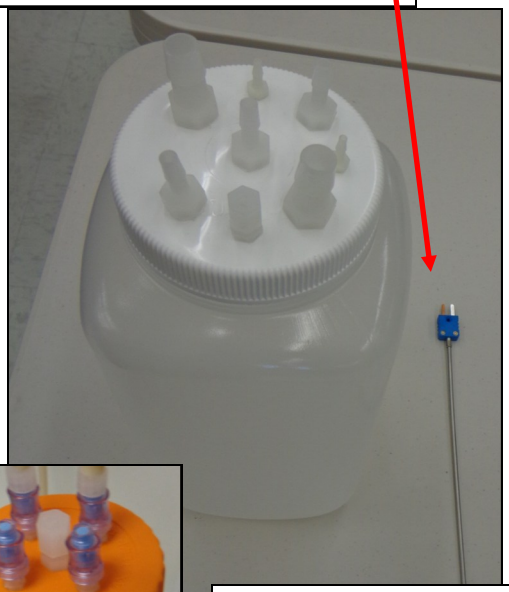
Power Cable
{31076 U.S.; 31077 Euro}
(see parts list for other countries)

ORCA4 Controller {32012}
ORCA3+1 Controller {32015}

Temperature Probe
{30960} 12" probe; $^{1}/_{16}$" diameter
{30959} 6" probe; $^{1}/_{16}$" diameter
{31079} 12" probe; $^{1}/_{8}$" diameter
{31078} 6" probe; $^{1}/_{8}$" diameter

Heater & Reservoir





Heater
{30250} 110V
{30247} 220V

Liner {31096}

Reservoir Sizes
{31105} **500mL**
{31104} **1000mL**
{31100} **2000mL**
{31099} **4000mL**

Note: 1/8" diameter temperature probes use a 1/8" compression fitting while 1/16" diameter probes use a 1/16" Touhy Borst fitting {31059}

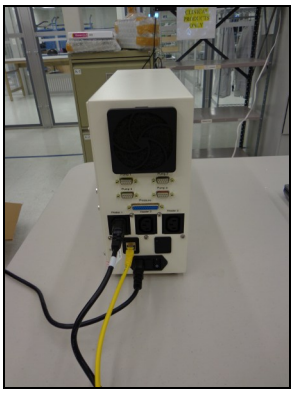
3.2 Assembling Components

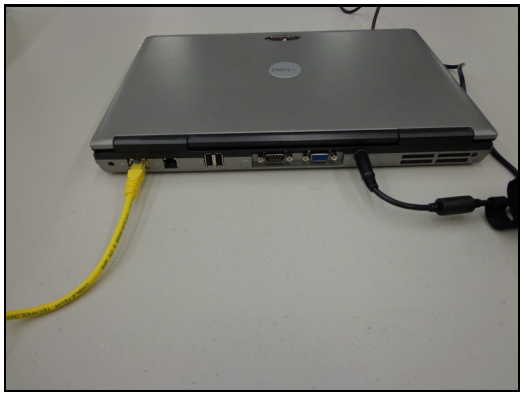
1. Place power cord into controller and electric source.

2. Connect laptop power cord to the laptop and electric source.

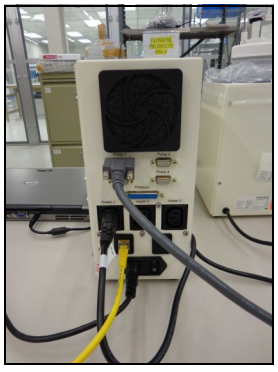
3. Connect Communications cable to laptop and ORCA Controller. (Cable color may vary.)

Note: In order to conserve valuable bench space, some users have found it useful to set the controller horizontally and to place the laptop on top of the controller.





4. Consider which pump to designate Pump #1, Pump #2, etc. The software will identify whichever pump is plugged into the top left port on the controller as Pump 1. It may be easier to identify the pumps if a label is attached to each drive motor (see image at bottom right). For each pump in use, connect pump communication cord to the back of the pump and to the corresponding location on the ORCA Controller.



Note: Some users stack pumps on top of another to save bench space. It is recommended that tubing be fed into the pumps prior to stacking.

5. Identify any extra pump heads and mounting screws that have been ordered. These may not be immediately needed for the system but should be kept in a safe place.

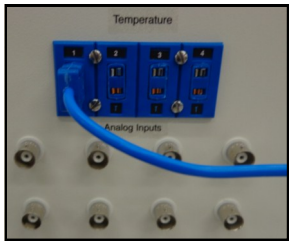
Pump heads {31108} Single Channel
{31107} Double Channel

Pump head **mounting screws** (mounting hardware for either 2,3 or 4 pump heads)

6. Connect heater power cord to the controller into heater slot #1. Add liner to heater and wrap it over the top of the heater. Then place reservoir bottle inside heater. Only the 4L bottle will fit snugly. An extension cable {31111} is available for order if more length is needed to connect the reservoir heater to the controller.

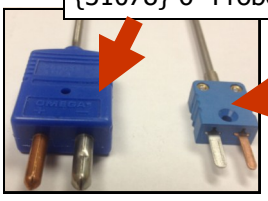
7. The smaller pronged end of the temperature probe adapters should be plugged into the appropriate port on the ORCA controller (find the labeled blue slots). For the reservoir heater, this is typically port #1. Connect the leads on the end of the thermocouple to the corresponding female slots on the adapter cable.

Note: Up to 3 probes can be used with an ORCA 3+1 controller (system with a pulsatile blood pump). The color on the probe connector should be matched with the same color on the ORCA controller panel: copper to copper and silver to silver.



{30961} Mini-T Adaptor

{31079} 12" Probe 1/8" OD
{31078} 6" Probe 1/8" OD

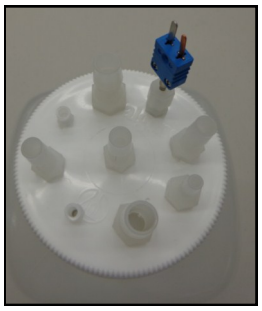


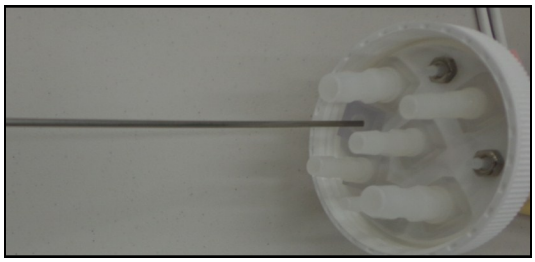
Probes with Mini-T connectors:
{30960} 12" Probe 1/16" OD Mini-T
{30959} 6" Probe 1/16" OD Mini-T
{31080} Intra-organ probe Mini-T

An extension cord may be needed. Check the probe head connector to determine if you need a large oval extension cable or the small slot extension cable #30961

8. Loosen the compression fitting then add temperature probe to reservoir. Be sure not to have the probe touch the bottom of the reservoir.

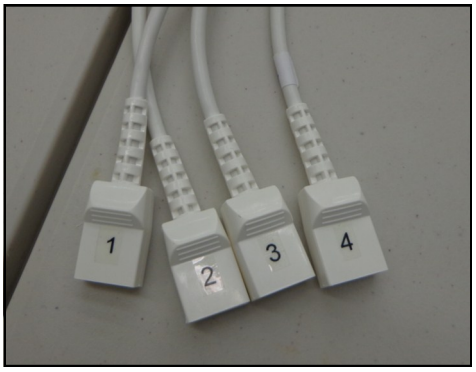
Insert additional probe into chamber for monitoring temperature within the chamber. For internal organ temperature measurements, a flexible implantable probe {31080} can be utilized.

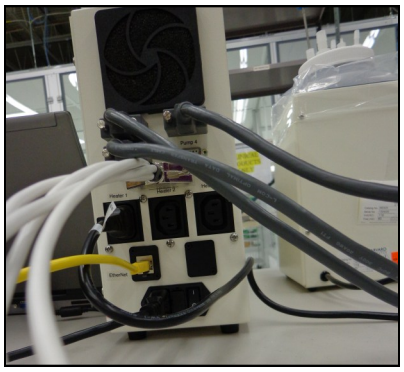




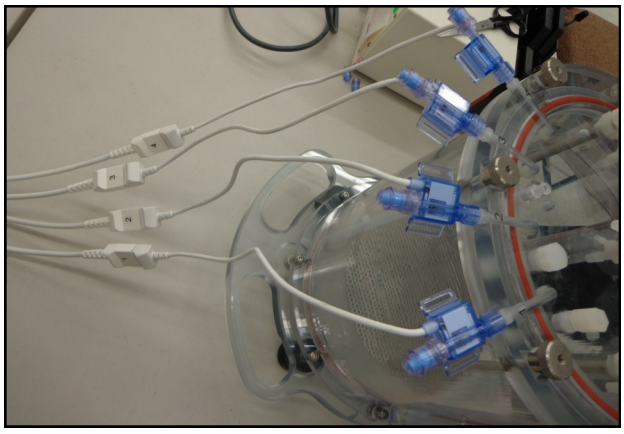
Note: Refer to the FAQ section of the manual for more information regarding temperature probes.

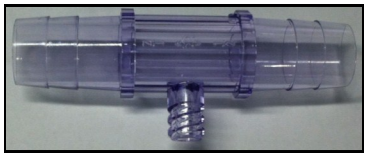
9. To set up pressure readings, mark the cables on the connector with the number of the channel that they represent (if not already labeled). Connect the other end of the pressure transducer cables to the ORCA controller.





10. When the system is to be used, the 4-to-1 transducer cable will be connected to each of the pressure transducers (see Operations section for calibration procedure). It is necessary to apply a lot of pressure when screwing the Pendotek pressure transducers into the side ports on the chamber lid. There are 3 side ports on the 5.5" chamber; there are 4 side ports on the 10" & 14" chambers. A swabable port should be attached to each of the transducers to maintain sterility (see bottom left image).



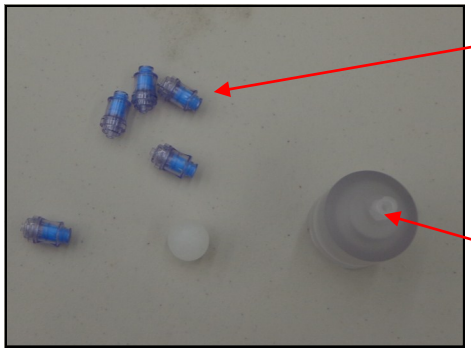


Straight Fittings w/ Luer Port for Pressure Transducers:
1/4" {30904}
3/8" {30899}
1/2" {30905}

Note: Make sure to clean transducers thoroughly before use. Pressure transducers are NOT autoclavable. Some media types have proteins and other materials that can nonspecifically bind to the pressure sensor and adversely affect the pressure readings. One technique used to minimize this effect is to inject 3 mL of sterile water through the swabable port. This provides an insulating fluid between the transducer and the medium without a negative effect on the pressure readings.

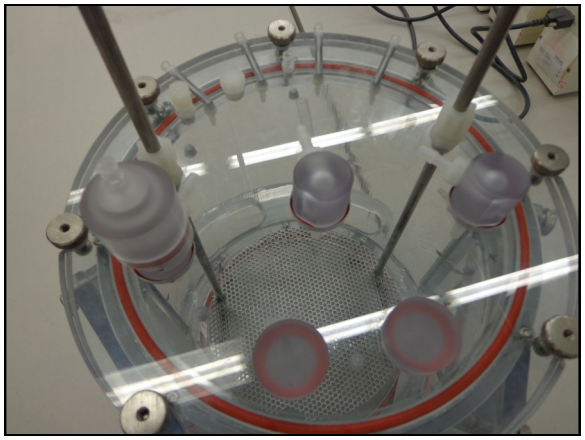
11. If not already assembled, place ball on bubble trap base, then place bubble trap head on base. The actual location of the bubble trap(s) may vary according to the system being used. The location of the bubble trap (and number required) as well as the right angle fittings may vary depending on the protocol being implemented.

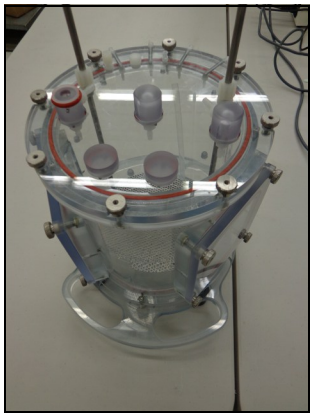
Note: In order to install the right angle fittings the bottom fitting must first be disassembled in order to fit through the Lid hole.

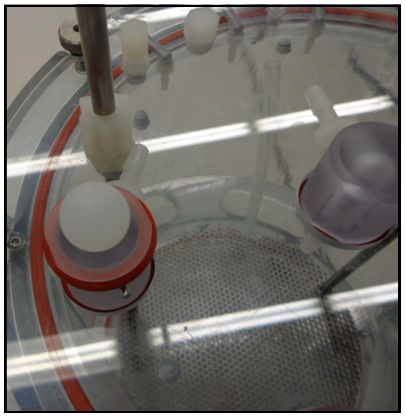


Swabable ports {31081} pk 25.
ONLY AUTOCLAVE ONCE to maintain integrity.

Bubble Trap Head
14" chamber {30297}
10" chamber {30450}



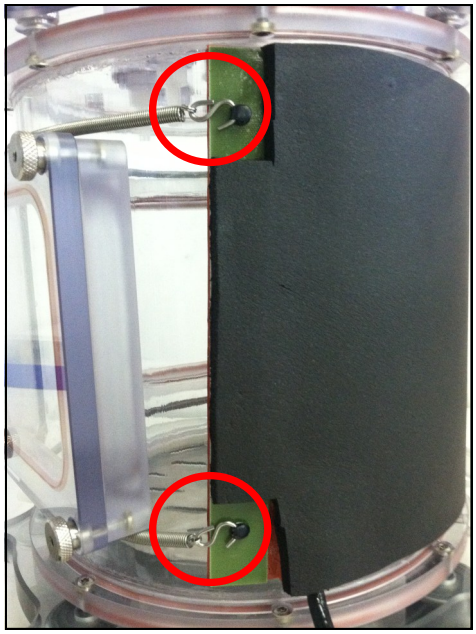


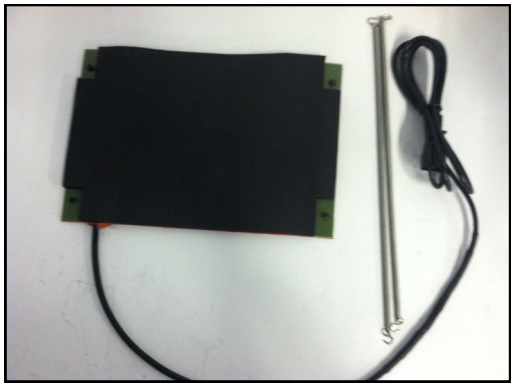


12. Attach the blanket heater to the chamber.

For 10" & 14" Chambers:

a. Place the heater pad on the back of the chamber.
b. Connect the springs to the hooks.





Blanket Heater Assembly
for 10" & 14" chambers
14" chamber 110V {31097}
14" chamber 220V {31195}
10" chamber 110V {31194}
10" chamber 220V {31144}

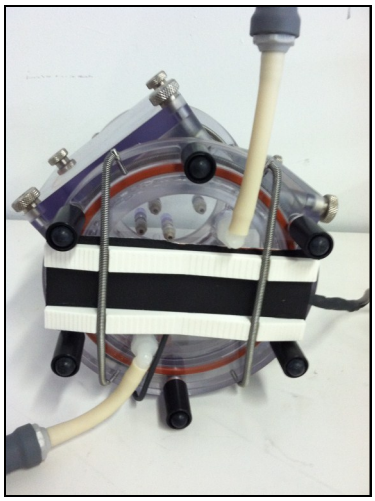
For All Chambers:

Set up the PID Controller for all blanket heaters in use. Connect the heater power cord into the PID Control box. Connect the temperature probe into the PID Box.

Note: Refer to Appendix B for more information about PID controller theory and application.

For 5.5" Chambers:

a. Orient the bottom heater pad with the power in cable so as to fit between the feet and drain valves.



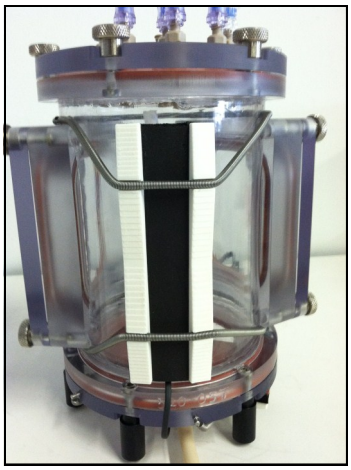
Blanket Heater Assembly
for 5.5" chambers
5.5" chamber 110V {31048}
5.5" chamber 220V {31193}

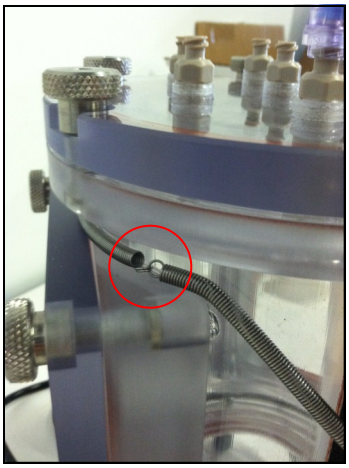
b. The heater pad (cord coming from the bottom) should be oriented so it will come up the back of the chamber.

c. Attach the two short springs between the metal pegs (dowel pins sticking out of the bottom plate).

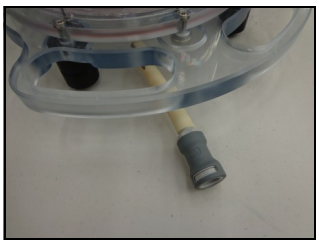
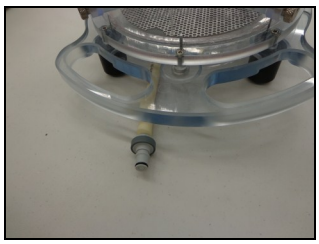
d. Wrap the 2nd heater pad against the back of the chamber and attach with the two long springs through the grooves in the heater.

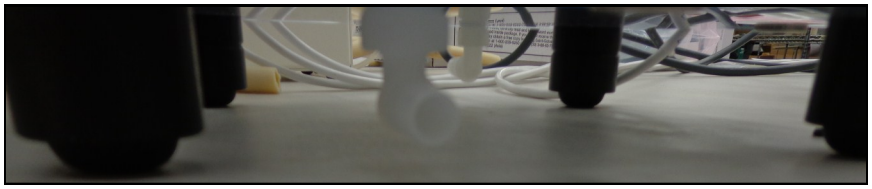
e. Fasten the springs by placing the spring hook into the loop.



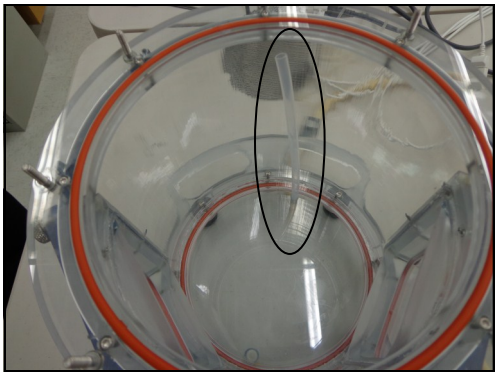
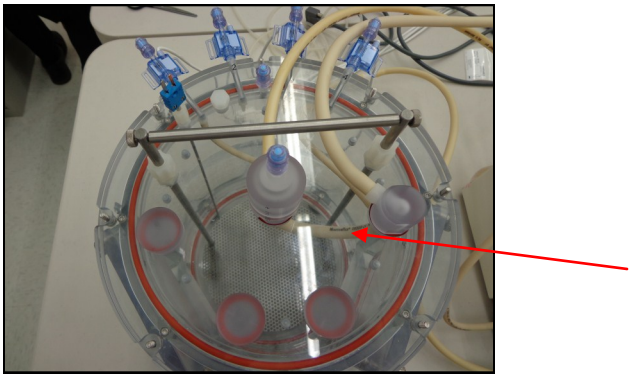


13. There are two fittings on the bottom of the chamber that allow filling and draining of the chamber. Add Quick Disconnects to facilitate the connections using an appropriate size of tubing from the chamber fitting.



14. The level of liquid in the chamber can be determined by cutting the rigid Teflon tubing inside the chamber to the desired height. Depending on the protocol, the user may want to cut the drain tube at an angle, with the low end of the cut toward the chamber wall, then file the point so that it is not sharp.

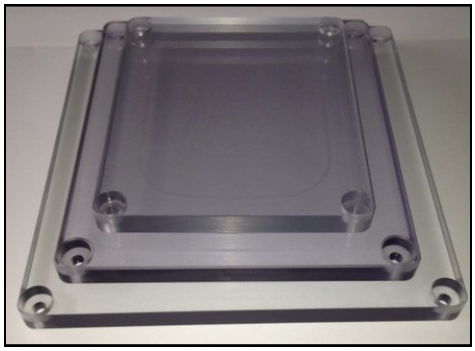
Note: If the user wishes to set up a complete flow path without having to sacrifice an organ, then a piece of tubing may be added in the place indicated (see above right image) to test the unit without an organ in place.

Note: The polycarbonate chamber windows (shown below for the 5.5”, 10”, and 14” chamber, respectively) are durable but can be prone to getting scratched. If the windows become too scratched, then replacements may need to be ordered. The replacement part numbers are shown below.

5.5” - {31083} includes gasket

10” - {31084} includes gasket

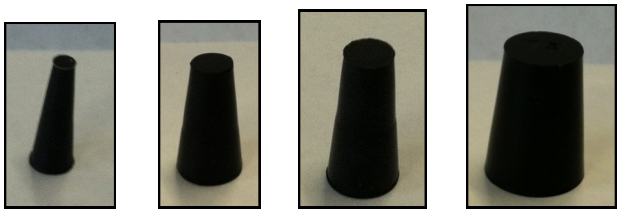
14” - {31085} includes gasket



15. Add appropriate size and length of tubing to the reservoir inlets.

Note: It is recommended that commonly used sizes of tubing are glued to a plastic clip board. For each size of tubing, cut a 1" piece and glue laterally as well as a ¼" piece glued so that the internal ID of the tubing is easily displayed. This is useful because otherwise it is often difficult to tell the correct size tubing needed at a glance. See page 22 for a reference guide.

16. Any barb fittings that are not in use should be sealed with the appropriately sized caps in order to maintain the system's sterility. There are 3/8", 1/2", and 3/4" caps available. They are shown below.



1/4" Plug, pk 10 {31112}
3/8" Plug, pk 10 {31113}
1/2" Plug, pk 10 {31114}
3/4" Plug, pk 10 {31115}

Pump Head Config.	Typical Function(s)
Single Channel Head	• Perfusion into organ at a low/medium flow rate
Two Single Channel Heads combined by a Y barb	• Perfusion into organ for smooth high flow rate • Empty chamber to reservoir
Four Single Channel Heads combined by a Y barb	• Highest flow rate
Two Dual Channel Heads (4 channel)	• Flow to oxygenator • Flow to pre-organ O_2 sensor • Flow to post-organ O_2, CO_2, pH sensors • Flow from a medium container
3rd Head	• May be added to the pump with two dual channel heads to allow addition and removal of reservoir medium.
4th Head	• Maximum flow rate (1.2 l/min = total of 4.8 l/min)

Tubing

Sizing ID	Sizing OD	PharMed Opaque, flexible		Tygon Clear, flexible		Teflon Translucent, more rigid	
1/32" (0.8 mm)	5/32" (.156 mm)		**L/S #13** 31101				
1/16" (1.6 mm)	3/16" (.188 mm)		**L/S #14** 31102				
1/8" (3.2mm)	1/4" (.25 mm)	A	**L/S #16** 30340	B	**L/S #16** 30341		
	1/8" (.318 mm)					C	30358
3/16" (4.8mm)	5/16" (.313 mm)	U	**L/S #25** 30339				
1/4" (6.4mm)	3/8" (.375 mm)	D	**L/S #17** 30338	E	**L/S #17** 30342		
	1/4" (6.4mm)					F	30345
5/16" (7.9mm)	7/16" (11.1 mm)	G	**L/S #18** 30337	H	**L/S #18** 30343		
	3/8" (9.5mm)					I	30346
1/2" (12.7mm)		AN	30552	J	30344		
	1/2" (12.7mm)					K	30347
3/4" (19.1mm)			30553				

Clamps

L	1/8" clamp	(25 ea)	31087
M	1/4" clamp	(25 ea)	31088
N	3/8" clamp	(25 ea)	31089
O	1/2" clamp	(25 ea)	31091

Connectors

P	1/8" connector	(10 ea)	31092
Q	1/4" connector	(10 ea)	31093
R	3/8" connector	(10 ea)	31094
S	1/2" connector	(10 ea)	31095
AK	Connector, 3/8 NPT - 3/8" Barb right angle	(10 ea)	31127
AM	Connector, 3/8NPT - 3/8" Barb	(10 ea)	31131

Fittings

V	Luer Lok Fitting	(10 ea)	31136

Y Barbs

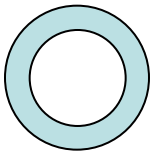
	1/4" Y barb	(10 ea)	31124
X	1/4" Y barb	(10 ea)	31125
Y	3/8" Y barb	(5 ea)	31126

Misc.

Z	Bubble Trap 1/2" (14" chamber)		30297
AA	Right Angle Fitting (14" chamber)		30420
AB	1/2" Universal Plug		30410
AC	1/4" Barb—1/2" NPT	(25 ea)	31128
AD	1/8" Barb—1/4"-28 UNF	(25 ea)	31129
AE	Bubble Trap 1/4"(10" chamber)		30450
AF	1/4" barb - 1/8" Right angle	(25 ea)	31132
AH	1/4" barb - 1/4" NPT	(10 ea)	31133
AI	1/8" pipe - 1/8 NPT Adapter	(10 ea)	31134
AJ	Female Luer 1/4-28	(10 ea)	31136
W	Swabble Port	(25 ea)	31081
AP	Pulsatile Pump head		31150
AR	Reducing Connector, 3/8 barb –1/4" Barb	(10 ea)	31137

PharMed Tubing Reference Guide

The following is intended to be a quick reference guide for deciding what size of PharMed tubing is currently being used. As previously mentioned, it is recommended that tubes of the sizes that your lab commonly uses are glued to a plastic clip board, allowing for even simpler comparison.

PharMed Tubing #	Front View	Tubing size	Lateral View
#13		0.15625” (4.0mm) **OD** 0.03” (0.8mm) **ID**	
#14		0.1875” (4.8mm) **OD** 0.06” (1.6mm) **ID**	
#16		0.25” (6.4mm) **OD** 0.12” (3.1mm) **ID**	
#25		0.3125” (7.9mm) **OD** 0.19” (4.8mm) **ID**	
#17		0.375” (9.5mm) **OD** 0.25” (6.4mm) **ID**	
#18		0.4375” (11.1mm) **OD** 0.31” (7.9mm) **ID**	
#82*		0.75” (19.1mm) **OD** 0.5” (12.7mm) **ID**	

* Used for connections—not for use in the peristaltic pump

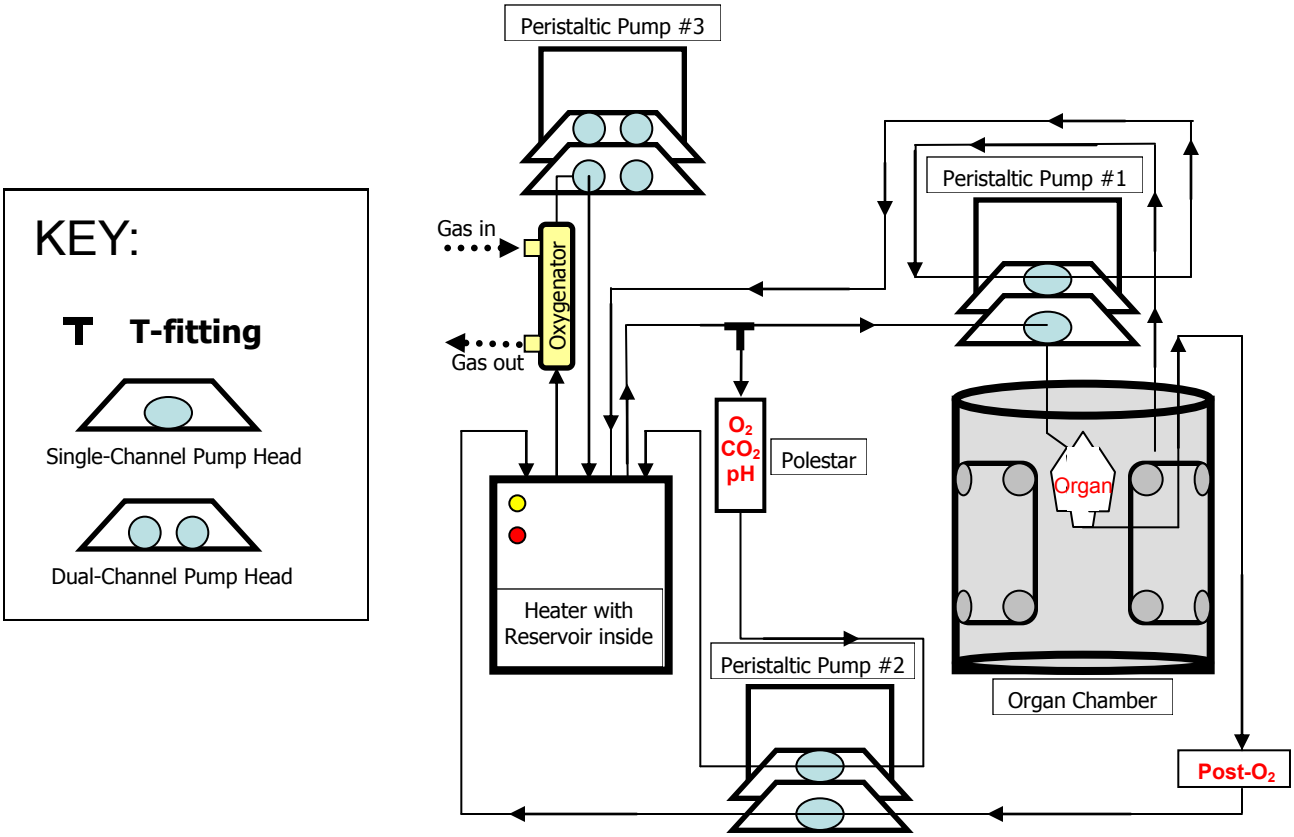
Note: When feeding the tubes through a peristaltic pump, it is recommended that you set a convention for flow direction that you follow for all pumps (i.e. forward flow is always designated as flow from left to right). This will help avoid potential confusion.

3.3 Chamber-Specific Flow Path Setup

i. 14” Chamber - Large Animal System

Example Configuration

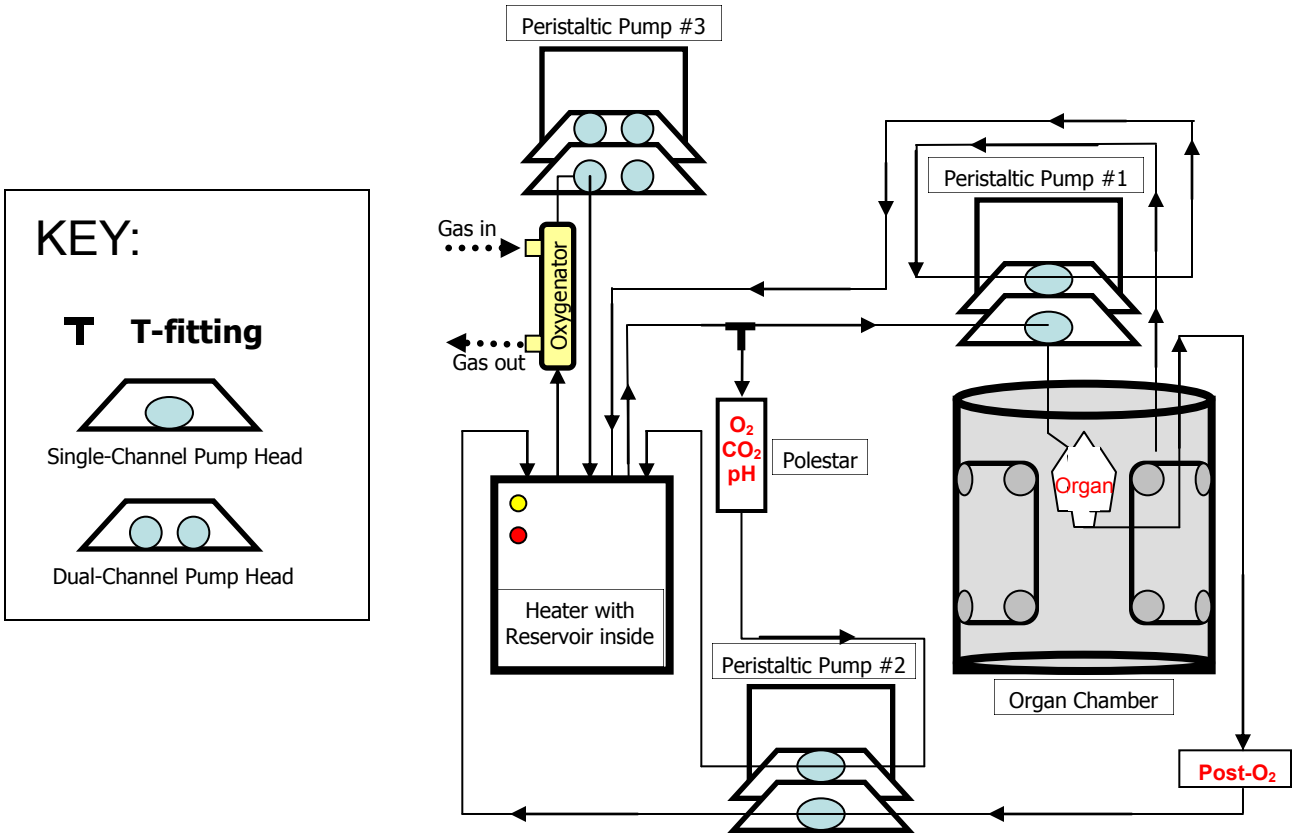
This is an example diagram representing a possible configuration for the ORCA bioreactor for a 14” chamber, large animal system. This is intended as a guideline to assist in setting up tubing pathways. Be sure to follow individual experimental procedures. Your laboratory’s setup may differ from what is depicted below; this diagram is not a replacement for your laboratory’s protocols.



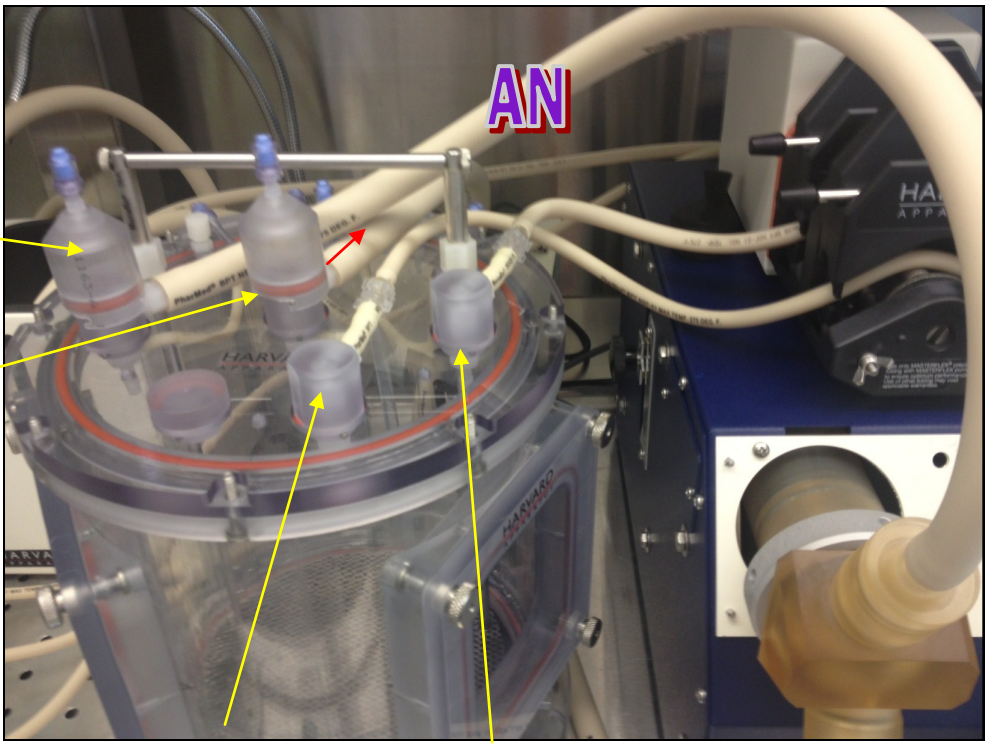
ii. 10” Chamber - Large Animal System

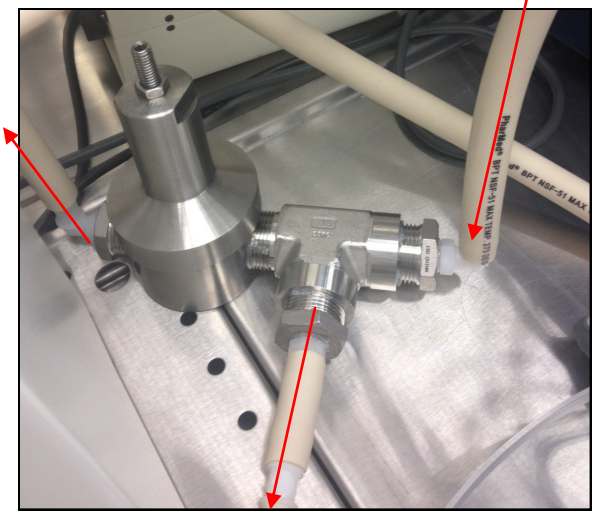
Example Configuration

This is an example diagram representing a possible configuration for the ORCA bioreactor for a 10” chamber, large animal system. This is intended as a guideline to assist in setting up tubing pathways. Be sure to follow individual experimental procedures. Your laboratory’s setup may differ from what is depicted below; this diagram is not a replacement for your laboratory’s protocols.

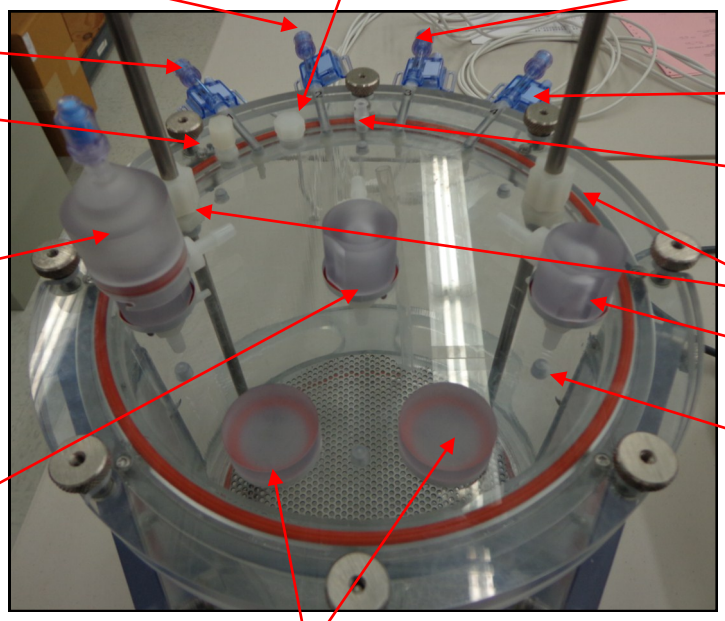


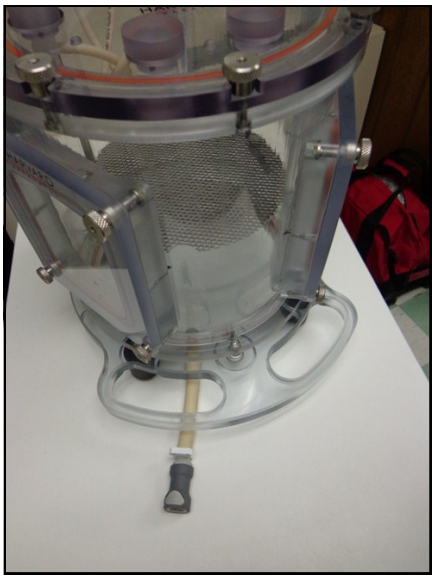
Large Animal Heart System - 10” Chamber





Large Animal Heart System - 10” Chamber

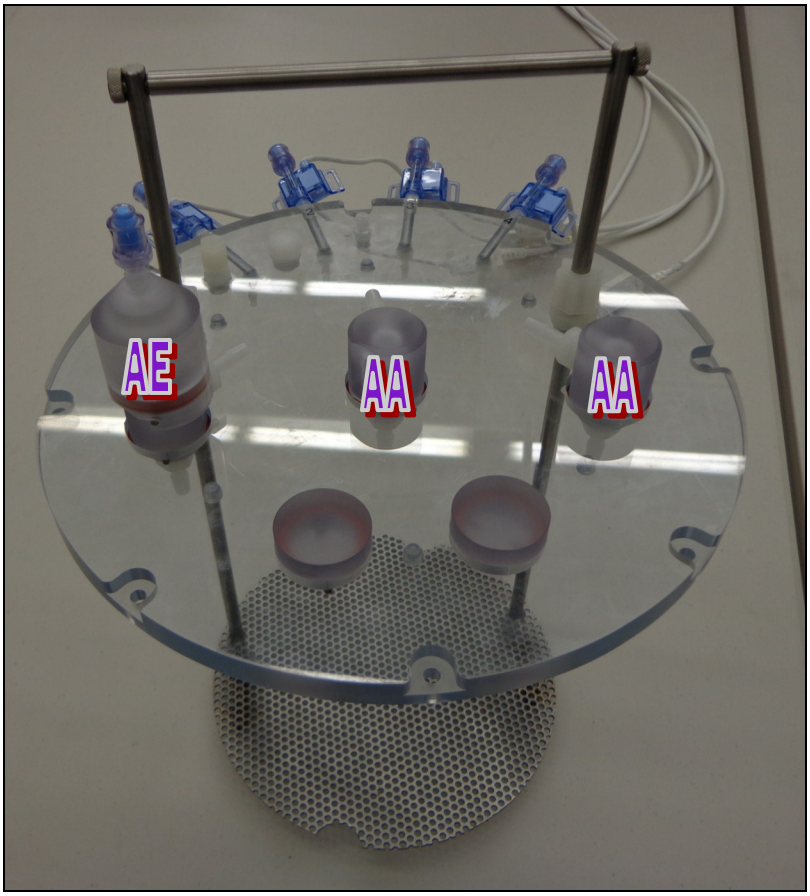




Chamber Quick Connect Fill
31211 $^{3}/_{8}$”
31210 $^{1}/_{4}$”

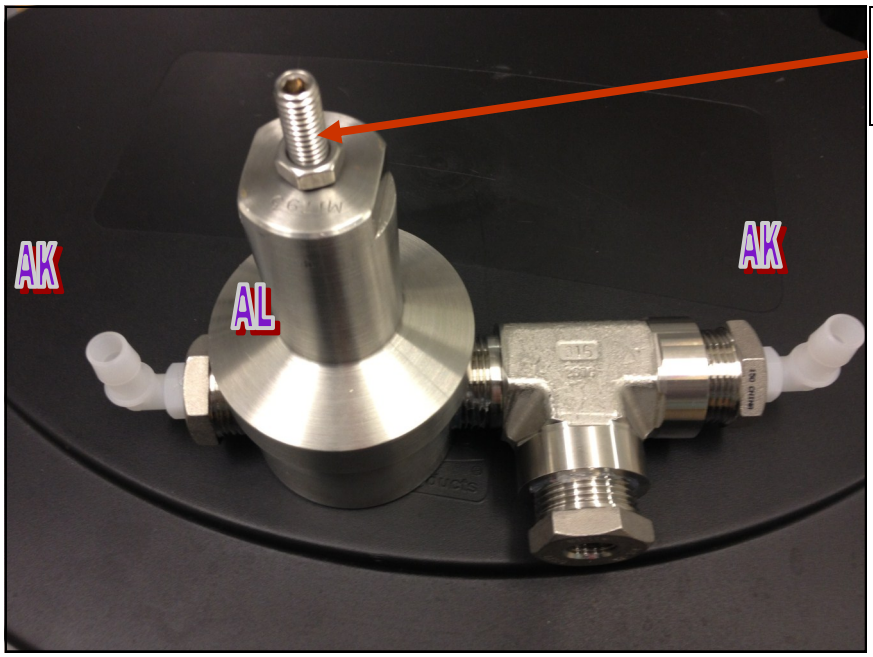
Chamber Quick Connect Drain
31211 $^{3}/_{8}$”
31210 $^{1}/_{4}$”

Large Animal Heart System - 10” Chamber

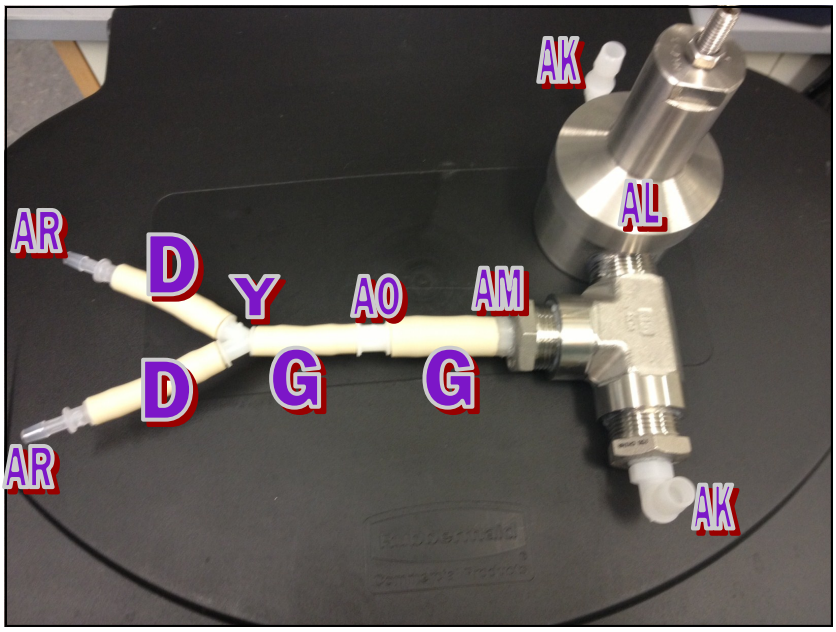


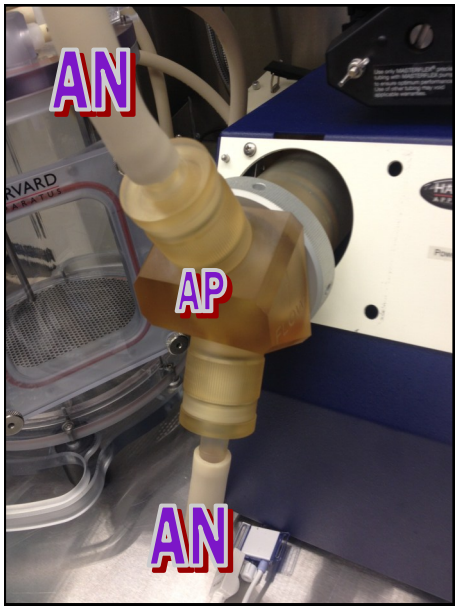
Large Animal Aortic Valve System - 10” Chamber

1/2” Diastolic after load pressure valve



Tighten (clockwise) to increase pressure

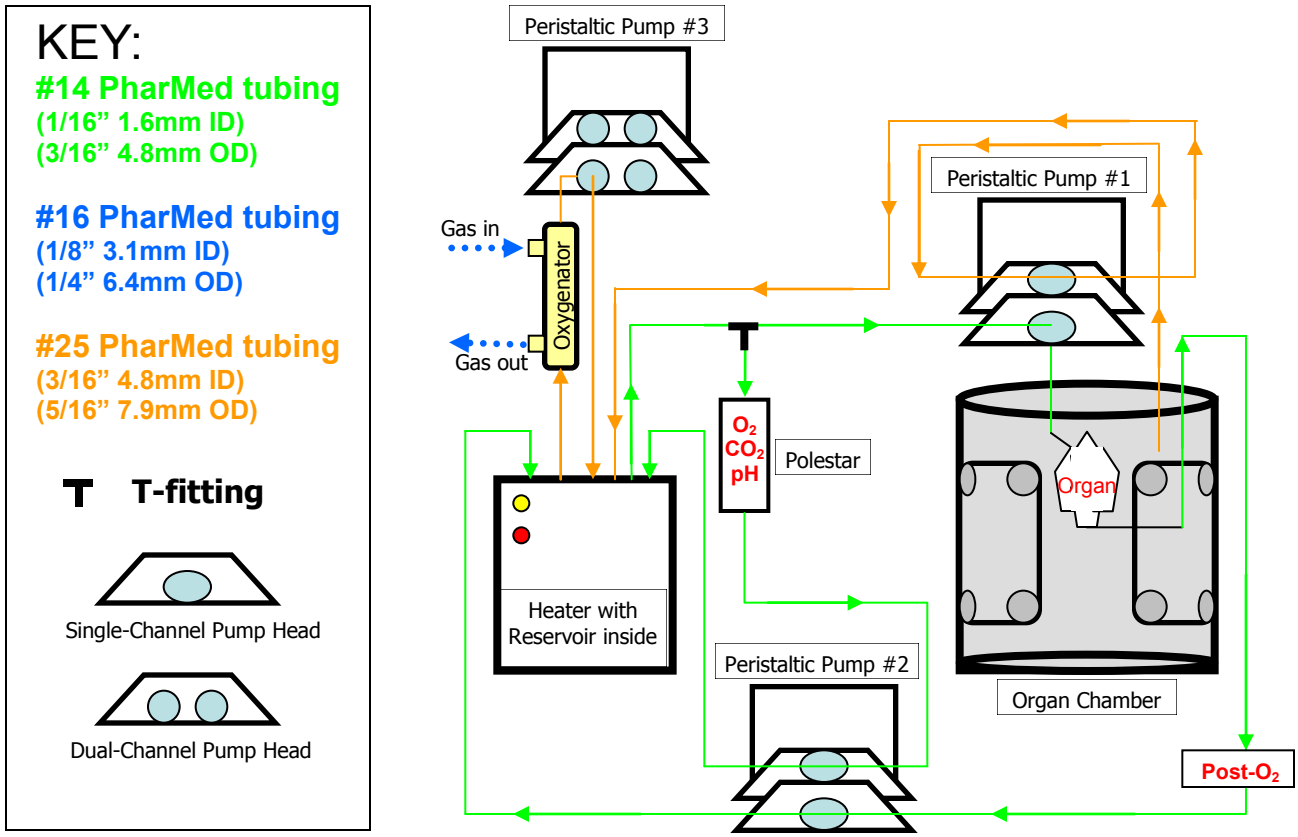




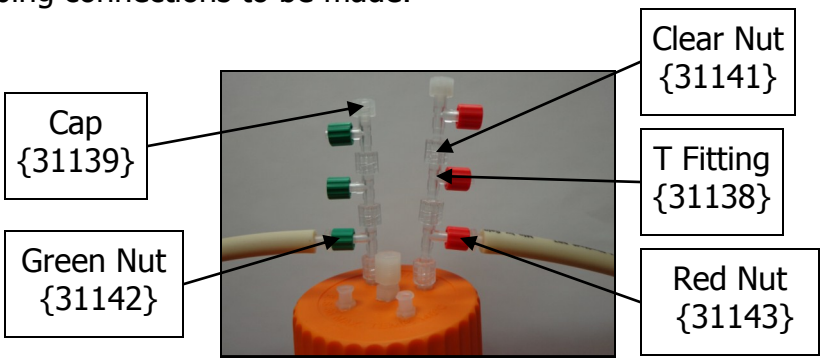
iii. 5.5” Chamber - Small Animal Lung System

Example Configuration

This is an example diagram representing a possible configuration for the ORCA bioreactor for a small animal lung system. This is intended as a guideline to assist in setting up tubing pathways. Be sure to follow individual experimental procedures. Your laboratory’s setup may differ from what is depicted below; this diagram is not a replacement for your laboratory’s protocols.

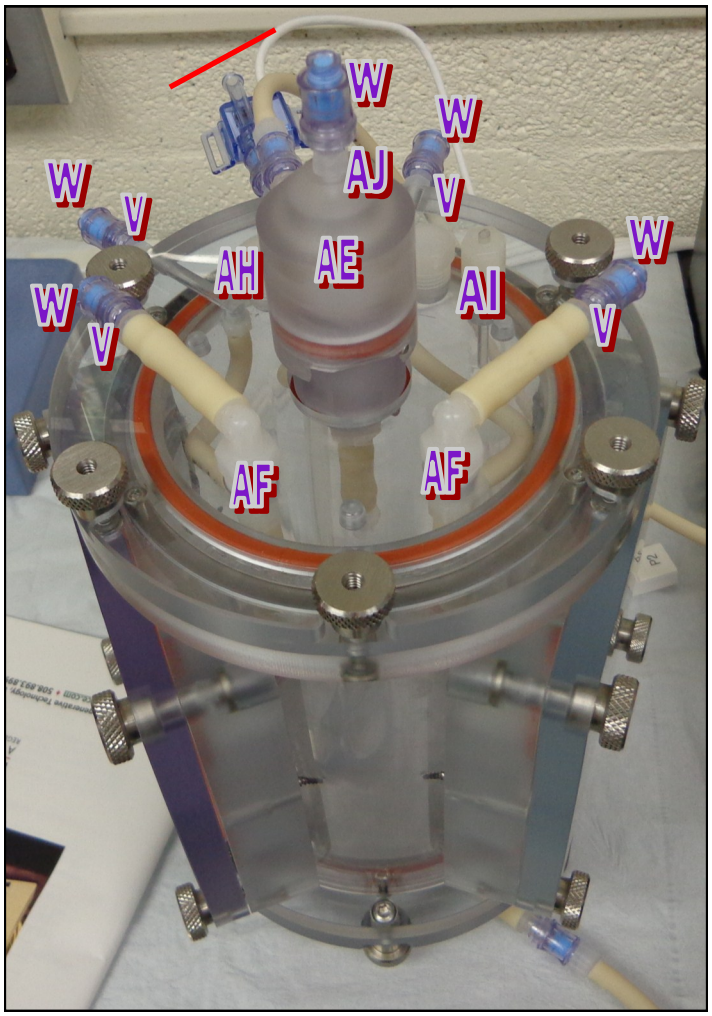


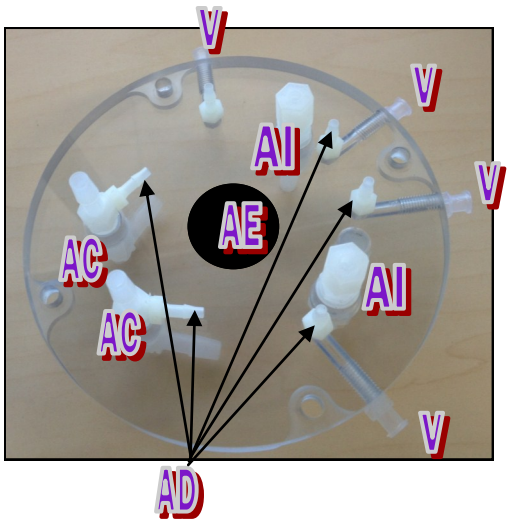
Inlet/Outlet Adaptor (for 500mL, 1L, & 2L Reservoir Bottle)
Allows more tubing connections to be made.

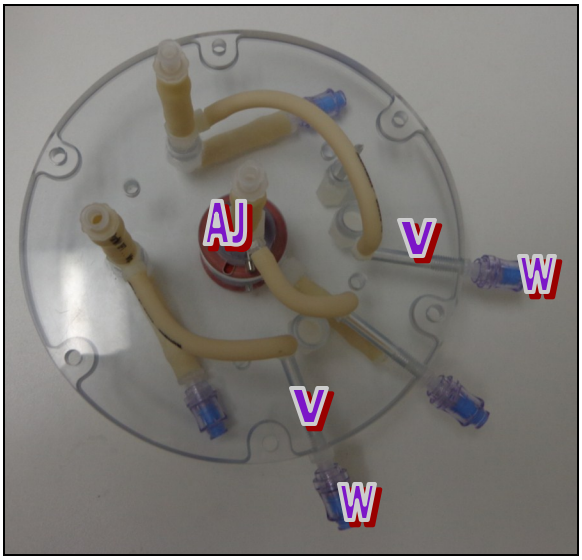


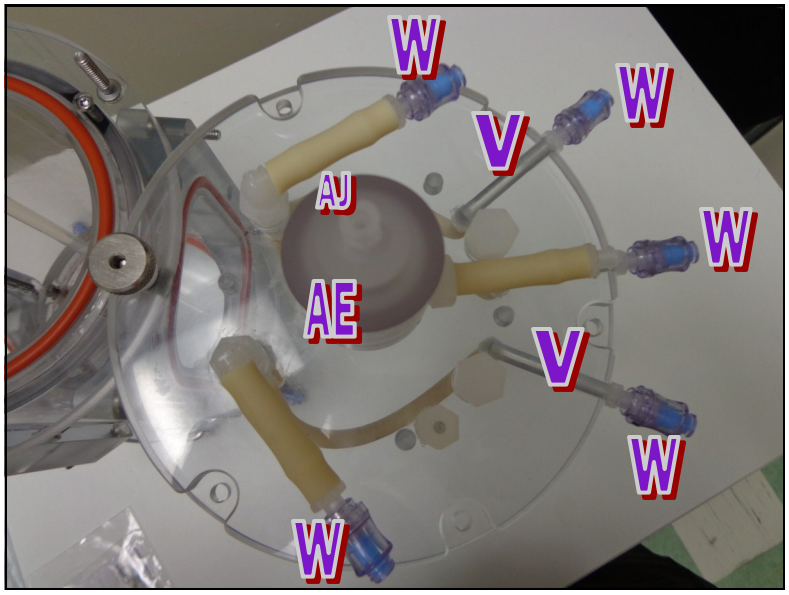
Note: Typically, green caps indicate inlets to the reservoir; red caps indicate outlets.

5.5” Chamber Lid

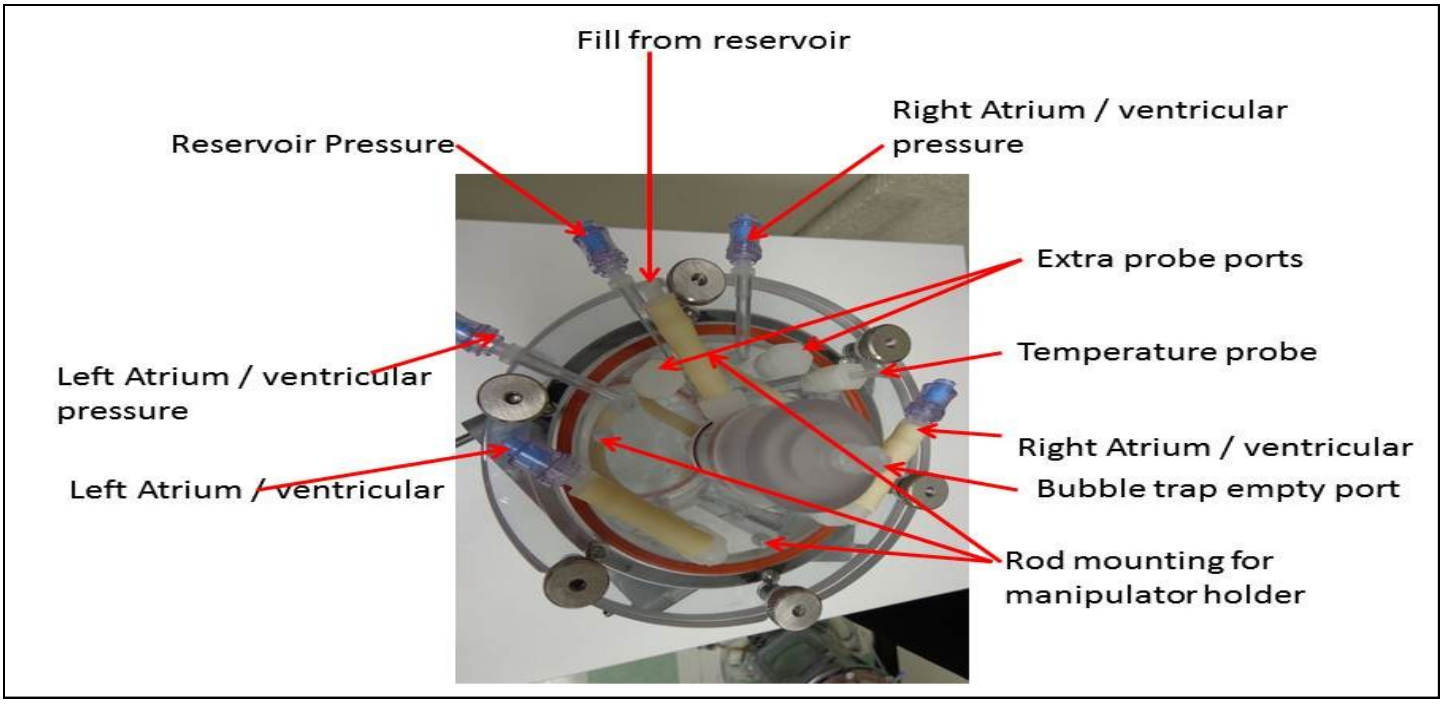




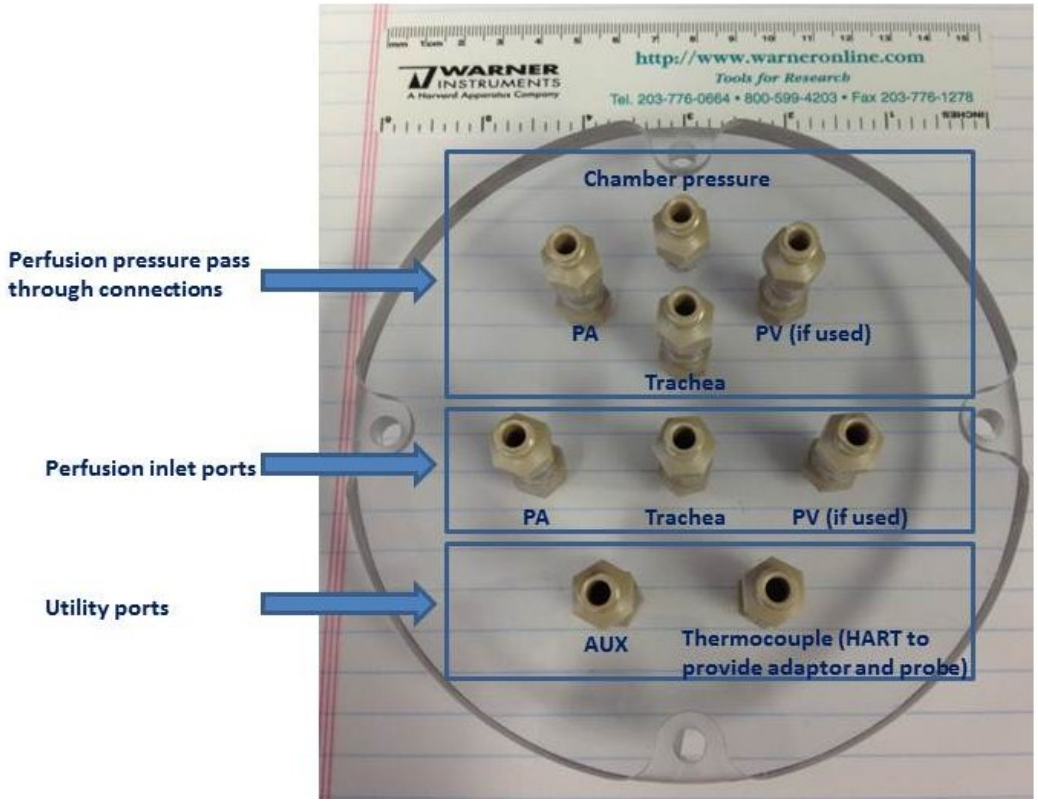




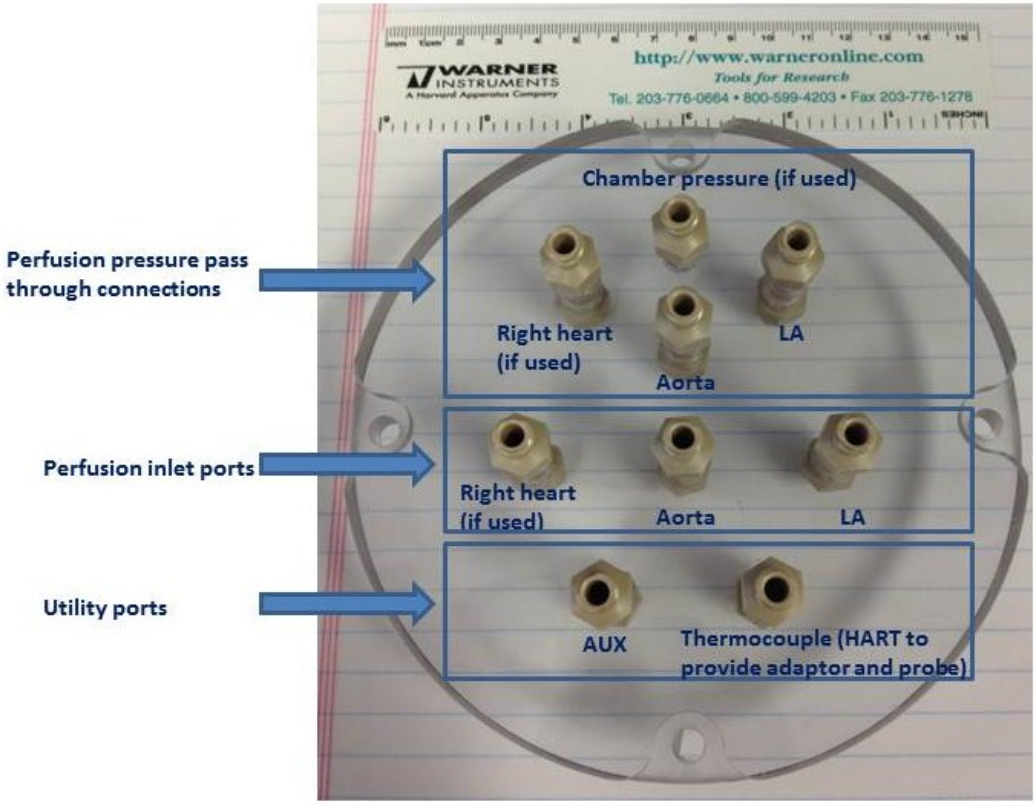
Mini Bubble Trap for 5.5” Chamber with Luer Fittings {31200}

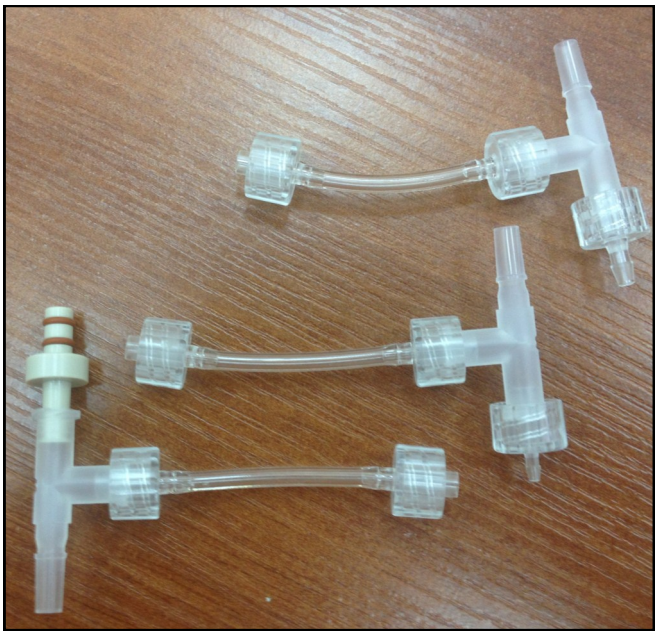

Fill from reservoir
Reservoir Pressure
Right Atrium / ventricular pressure
Extra probe ports
Temperature probe
Left Atrium / ventricular pressure
Right Atrium / ventricular
Left Atrium / ventricular
Bubble trap empty port
Rod mounting for manipulator holder

Lung

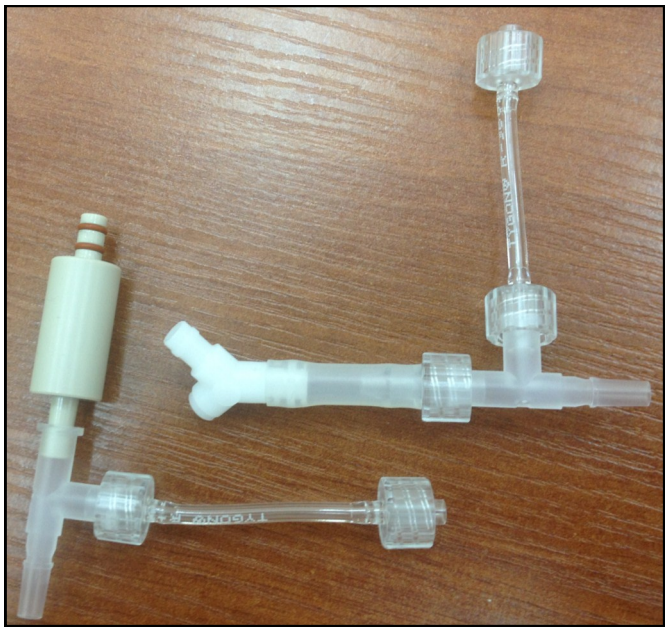

WARNER INSTRUMENTS
A Harvard Apparatus Company
http://www.warneronline.com
Tools for Research
Tel. 203-776-0664 • 800-599-4203 • Fax 203-776-1278
Chamber pressure
PA
PV (if used)
Trachea
Perfusion pressure pass through connections
Perfusion inlet ports
PA
Trachea
PV (if used)
Utility ports
AUX
Thermocouple (HART to provide adaptor and probe)

Heart


WARNER INSTRUMENTS
A Harvard Apparatus Company
http://www.warneronline.com
Tools for Research
Tel. 203-776-0664 • 800-599-4203 • Fax 203-776-1278
Chamber pressure (if used)
Right heart (if used)
LA
Aorta
Perfusion pressure pass through connections
Perfusion inlet ports
Right heart (if used)
Aorta
LA
Utility ports
AUX
Thermocouple (HART to provide adaptor and probe)



Cannula Adapter Kit
for 5.5" Chamber {31116}
(typically for rodent lung)



Cannula Adapter Kit
for 5.5" Chamber {31117}
(typically for rodent heart)

Cannulae are available in various sizes. Typical sizes include:

Mice	1 mm
Rat	2.3 mm
Rabbit	3 mm or 4 mm

Chapter 4: Operating Instructions

Prior to Starting

Assure that all the requirements identified in Chapter 2 have been successfully met.

Personnel using the ORCA Bioreactor should read through this manual in its entirety prior to using the device.

WARNING:

Failure to follow aseptic techniques and failure to train on all processes and procedures prior to using the bioreactor may result in critical delays, contamination, and other harmful events.

4.1 Sterilization

Sterilization can be performed using EtO or by autoclaving. **It is strongly recommended that all fittings be loosened prior to sterilization procedure.** Tubing has a tendency to deform around the barb during an autoclave cycle and can become loose and allow leaks.

One possible sterilization procedure would involve assembling the tubing set as it is to be used in the bioreactor and placing it into a sterilization bag to be autoclaved; the chamber could then be sterilized in a separate sterilization bag. Be sure to follow your laboratory's individual sterilization protocols.

It is also strongly recommended that PharMed tubing be used throughout the system. It has shown to be the most resilient to the intense heat and pressure of the autoclave.

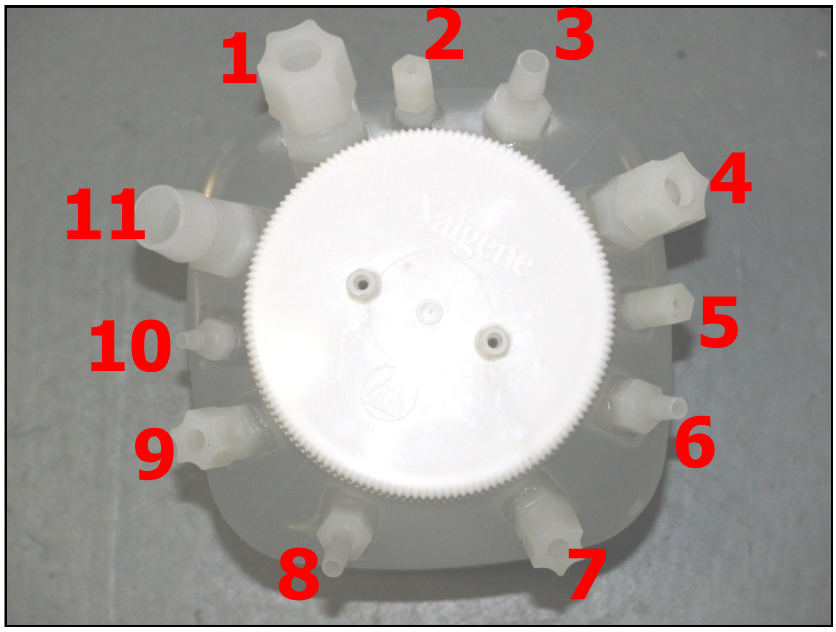
Tygon tubing is very susceptible to warping after autoclavation. If the flow path is autoclaved and has Tygon tubing, check all barb connections. In many cases Tygon tubing will become soft and may slide off the barb after autoclaving when pressure is applied. If the tubing is soft on the barb, it is recommended that the portion of the tubing that was on the barb is cut and discarded and that fresh tubing is slid onto the barb. Ty-wraps may also be used on barb fittings.

4.2 Reservoir Bottle Fittings & Typical Usages

The reservoir bottle for the ORCA Bioreactor comes in a variety of configurations. The various models are all displayed in the next few pages of this manual. Please find the model that was included in your laboratory's particular bioreactor package.

Large Animal

Note: This diagram depicts a typical usage for each of the reservoir bottle ports in a large animal system. Be sure to follow your laboratory's individual protocols.



1) 3/4" tube; Pulsatile for trachea

2) 1/8" tube

3) 3/8" rigid tube; PA on lung or pressure control for valve peristaltic

4) 3/8" tube; PA on lung or pressure control for valve peristaltic

5) 1/8" rigid tube

6) 1/4" rigid tube; chamber circulation

7) 1/4" tube; chamber circulation

8) 1/4" rigid tube; oxygenator circulation

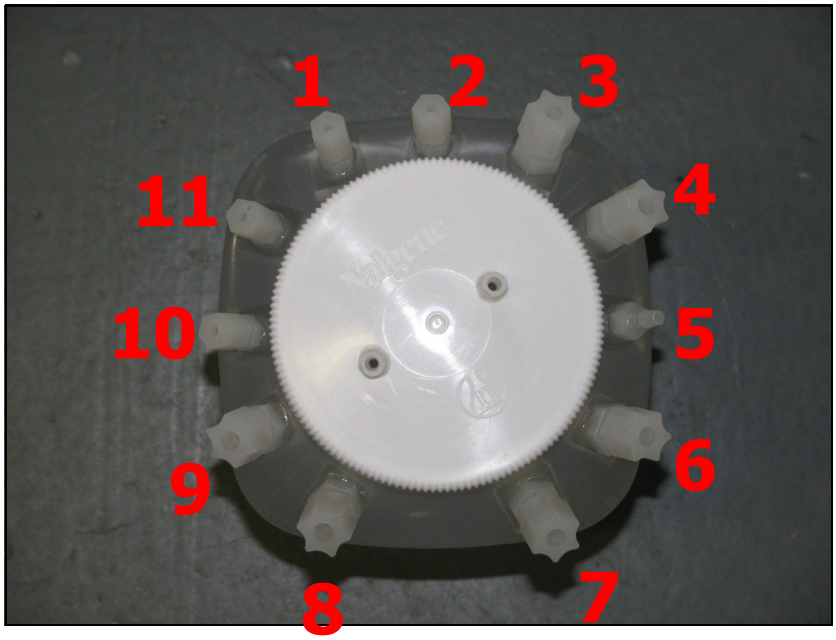
9) 1/4" tube; oxygenator circulation

10) 1/8"; thermocouple

11) 1/2" tube; Pulsatile for trachea

Small Animal

Note: This diagram depicts typical sizing & usage for each of the reservoir bottle ports in a small animal system. Be sure to follow your laboratory's individual protocols.



1) 1/8" tube

2) 1/8" rigid tube

3) 1/4" rigid tube; PA on lung or pressure control for valve peristaltic

4) 1/4" rigid tube; PA on lung or pressure control for valve peristaltic

5) 1/8" tube

6) 1/4" rigid tube; chamber circulation

7) 1/4" rigid tube; chamber circulation

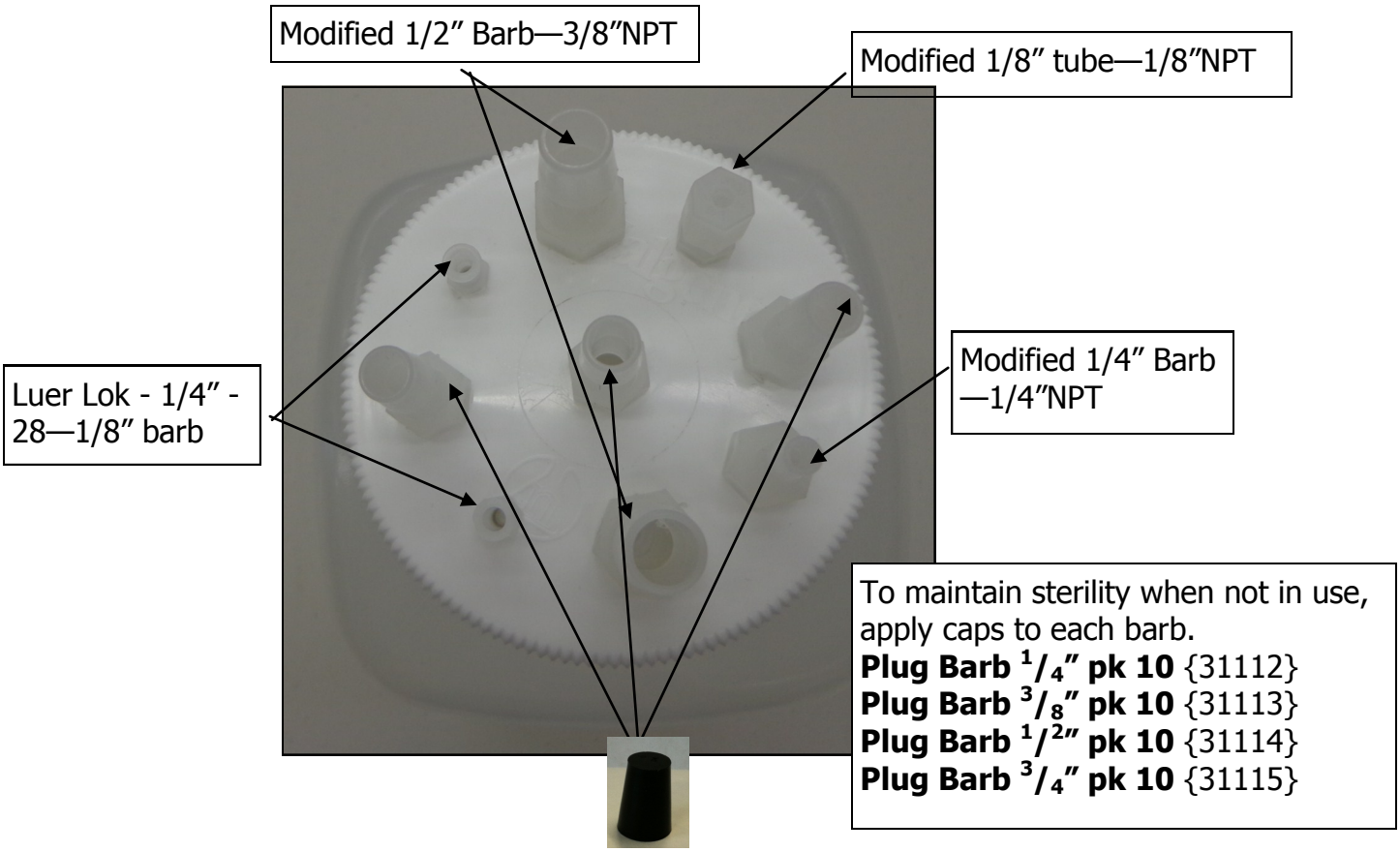
8) 1/4" rigid tube; oxygenator circulation

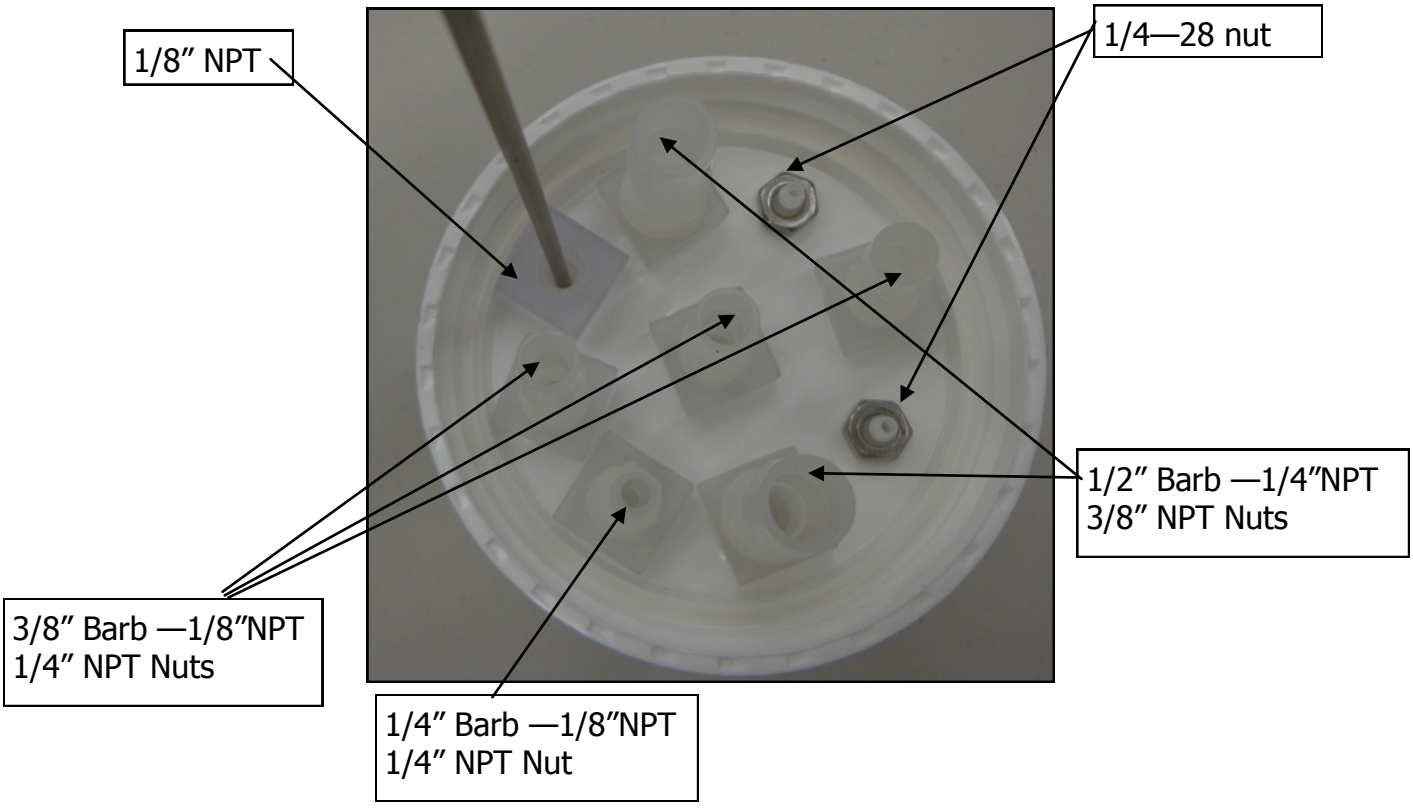
9) 1/4" rigid tube; oxygenator circulation

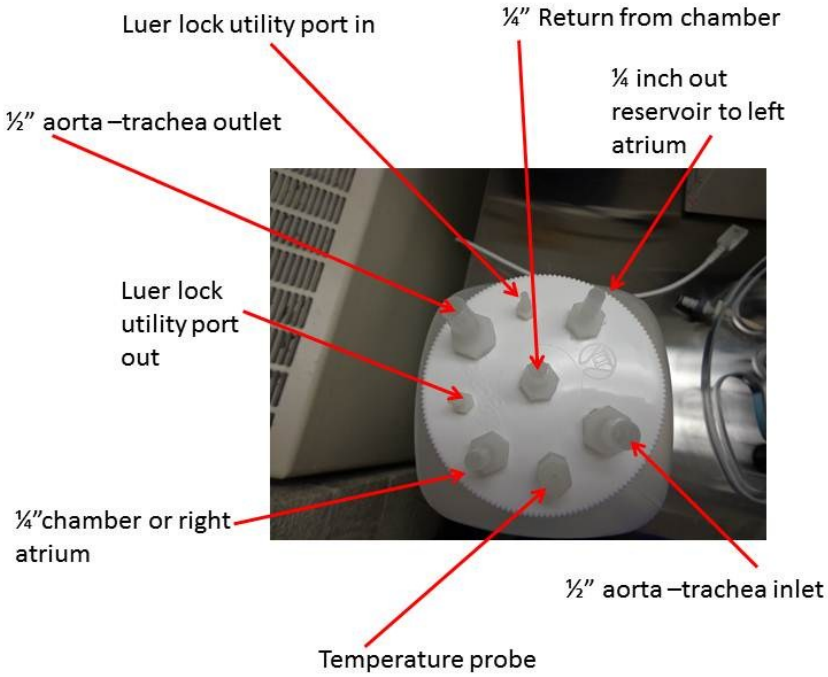
10) 1/8"; thermocouple

11) 1/8" rigid tube

Cap mounting Plastic Bottle Large Animal


Modified 1/2" Barb—3/8"NPT
Modified 1/8" tube—1/8"NPT
Luer Lok - 1/4" - 28—1/8" barb
Modified 1/4" Barb —1/4"NPT
To maintain sterility when not in use, apply caps to each barb.
Plug Barb 1/4" pk 10 {31112}
Plug Barb 3/8" pk 10 {31113}
Plug Barb 1/2" pk 10 {31114}
Plug Barb 3/4" pk 10 {31115}




Luer lock utility port in
¼" Return from chamber
¼ inch out reservoir to left atrium
½" aorta –trachea outlet
Luer lock utility port out
¼"chamber or right atrium
½" aorta –trachea inlet
Temperature probe

Manifold allowing multiple feeds or return lines may be created as necessary. Add the number of Slip Luer T-fittings as required.

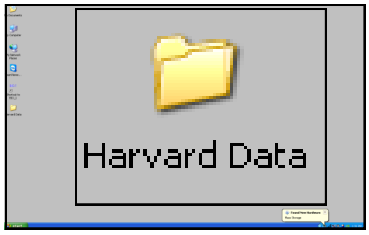
Polycarbonate products are autoclavable. They must be thoroughly rinsed before autoclaving because detergent residues cause crazing and spotting. Autoclaving cycles should be limited to 20 minutes at 121°C. PC shows some loss of mechanical strength after repeated autoclaving and therefore may not function well under high-stress applications, such as centrifugation.

4.3 ORCA Controller Software

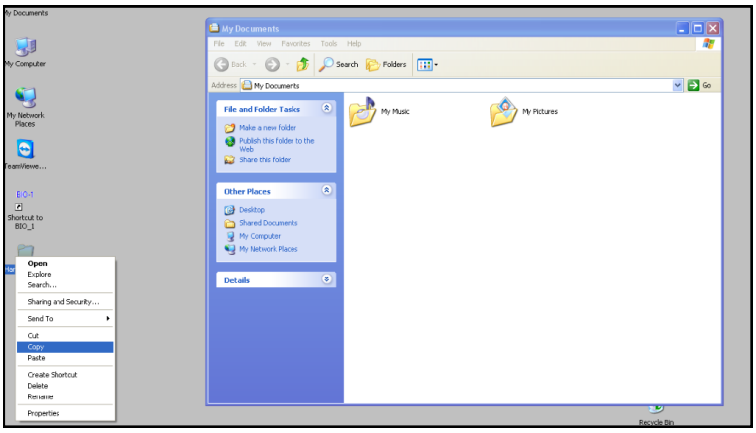
Operation Instructions

The operation software allows for control of flow rate and gives temperature, pressure, and flow rate readouts over time. The user can control a maximum of four pumps at once; both pulsatile and peristaltic pumps can be utilized.

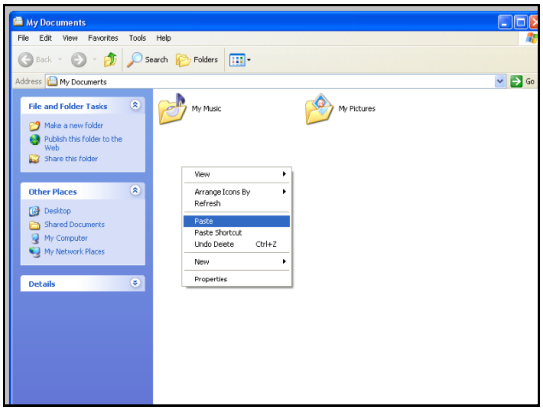
Before running the software for the first time, locate the folder "Harvard Data" on the desktop and copy/paste it into the My Documents folder as a backup. This ensures that a backup copy is available in case the program is ever shut down incorrectly. In recent systems, this will already have been done by HART prior to shipping to the user.



Click on the Harvard Data Folder on the desktop. Select "COPY".



Click on the My Documents Folder on the desktop. Select "PASTE".



Running & Terminating the Program

Be sure of the following before running the software:

1. Windows auto-updates is turned off, otherwise it could cause the computer to restart whilst running an experiment.
2. The ORCA power cord is plugged into the ORCA controller and the wall
3. The Communications cable is plugged into computer and into the ORCA controller
4. The ORCA controller is turned ON

To run the program, select the Bio_1 initiation icon from the Desktop. **When finished with an experiment, it is important to shut down the ORCA controller software using CTRL+Q or the "File --> Stop" option.**

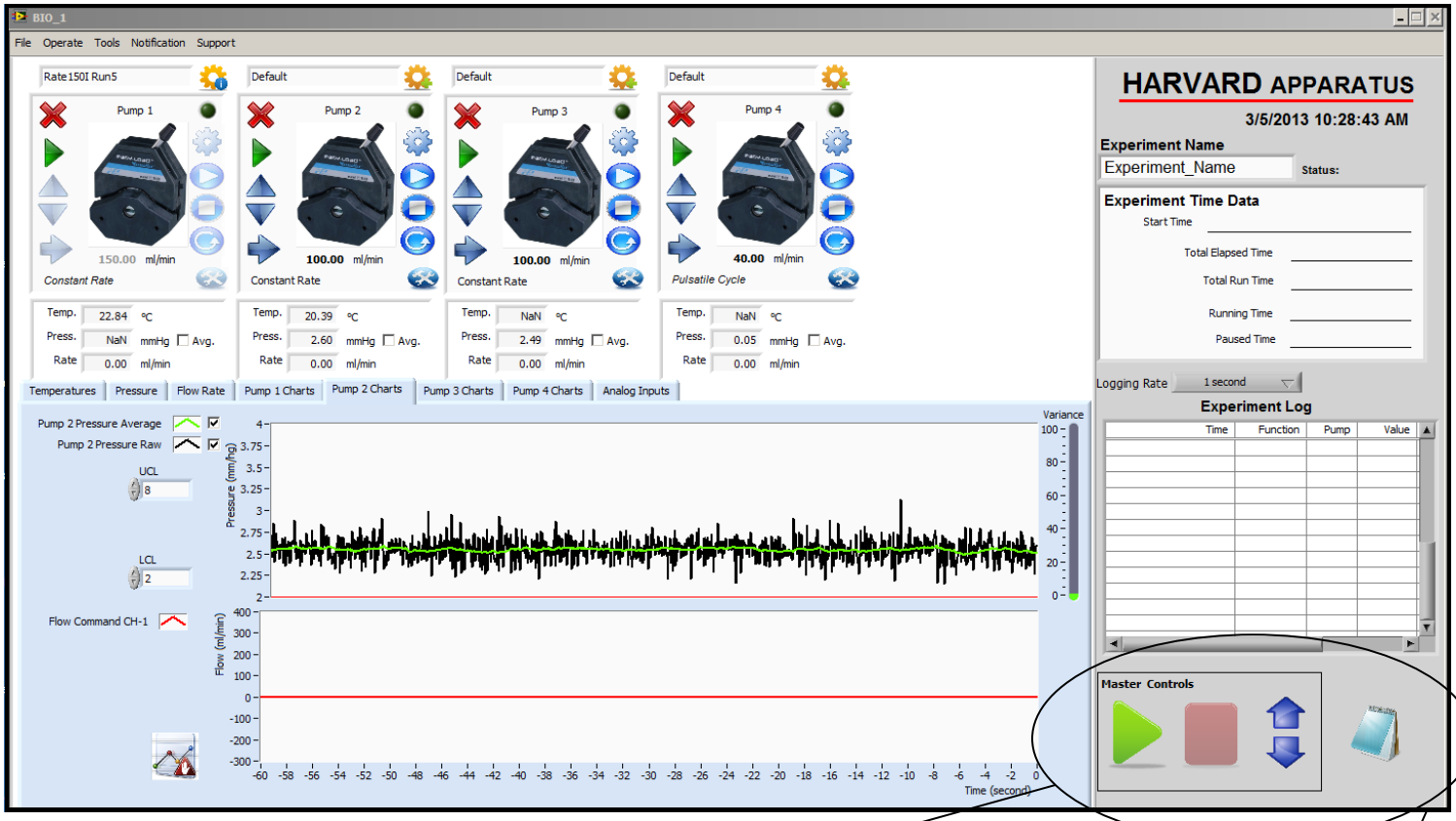
WARNING: Shutting down the program by shutting off the PC or using any other method may result in a corrupted CONFIGURATION FILE and not allow the program to initiate properly the next time it is used. As a result, the red X button normally found in the upper right corner of the screen has been disabled.

Improper Shut Down may corrupt the config file.

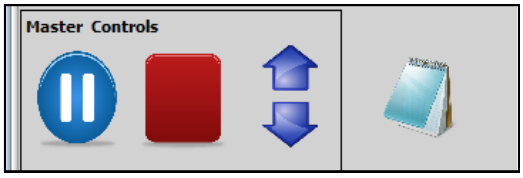
If the config file is corrupted, a back-up is available to correct the situation.

1. Open My Documents folder and delete the Harvard Data folder
2. Copy the "BackUp Harvard Data" folder into my Documents
3. Rename the "BackUp Harvard Data" to "Harvard Data"

The Main Screen



In the above state, no data logging is occurring. Clicking the green button will begin the program.



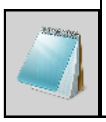
When the trials are running, the master controls will appear as above. Use the red button to stop all data logging.

Master Controls:

Starts all pumps.

Stops all pumps.

Incrementally adjusts flow rate of all pumps



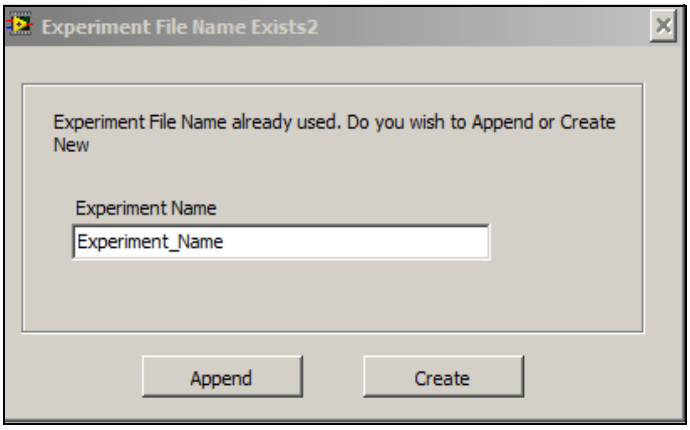
Adds an Experiment note.

Starting Pump Programs

The green icon starts the programs of all active pumps and begins data logging. Before data logging begins, the user is prompted to either create a new file or append.

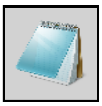
If the "create" option is chosen but the file name has already be taken, the program will issue a warning.

The disk icon indicates that you are collecting data.



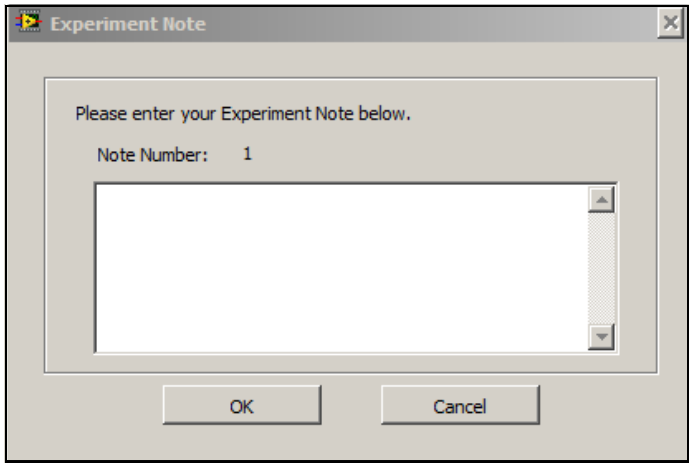
Experiment Log

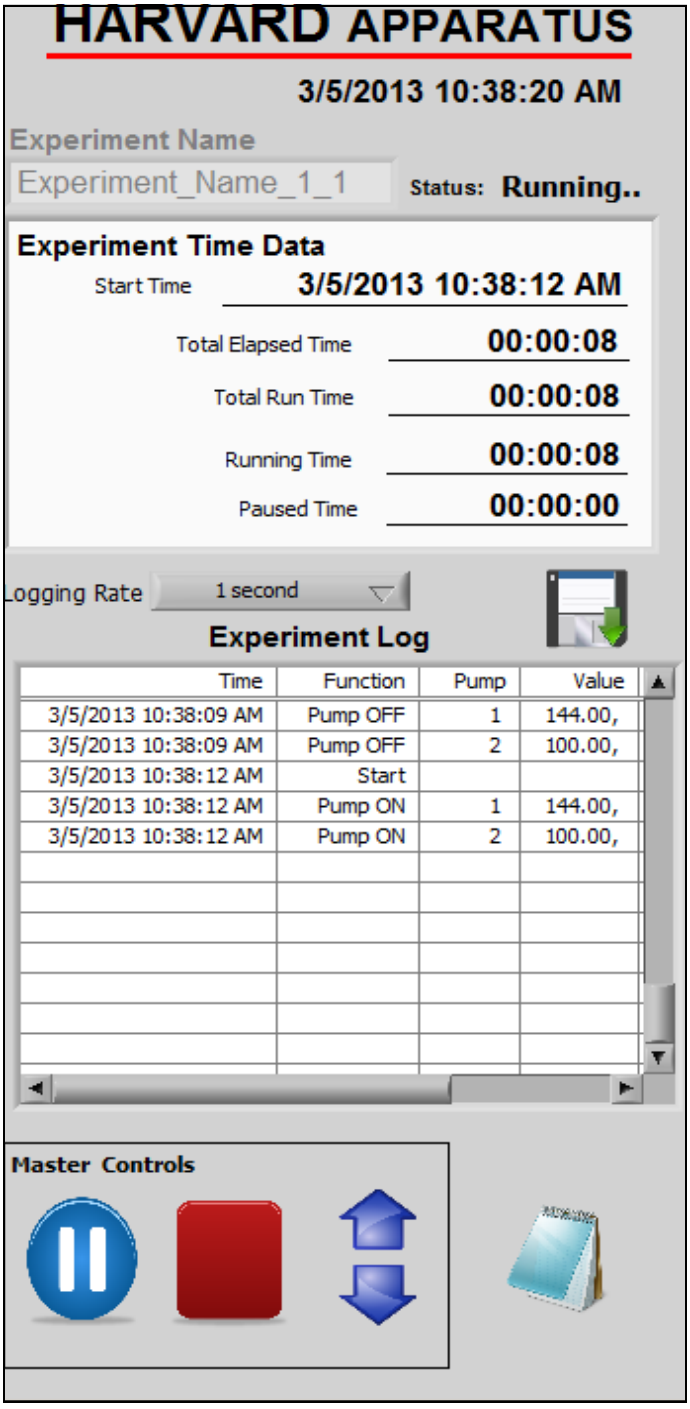
All data will be continuously entered into the experiment log until the user stops the run.



Experiment Notes

Clicking on the Notepad icon allows the user to enter an experiment note.





Enable/Disable Pump
This primes a pump for use or deactivates a pump when not in use.

Start/Stop Run
Starts or stops the corresponding pump.

Flow Rate Control
These up/down arrows manually adjust the flow rate while the run takes place. The flow rate can also be altered by clicking on "**x.xx ml/min**" just below the pump icon and entering a value.

Flow Direction
Changes the direction that the pump rotates; a right arrow indicates clockwise.

Create Step
Use this to create a Step. See "Step Creation" for additional info.

Select Step
Use this to select a Step. See "Step Creation" for additional info.

Green Light
Glows when pump is on.

Step Mode
See "Step Mode" for additional info.

Start Mode
See "Start Mode" for additional info.

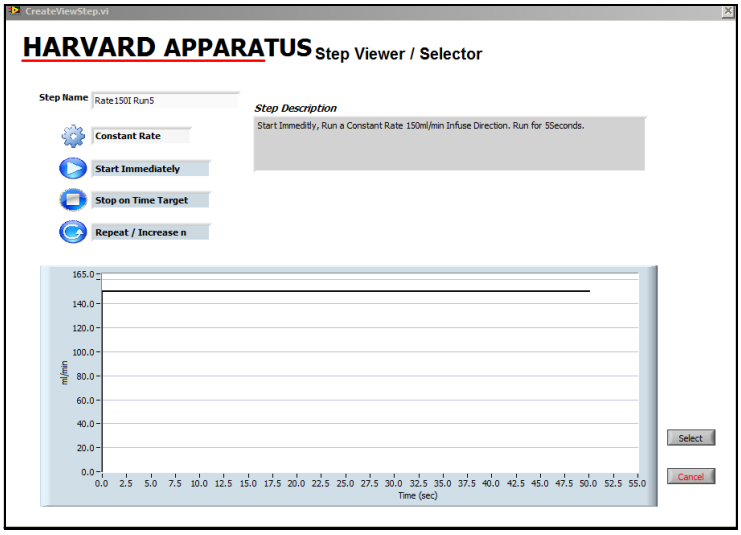
Stop Mode
See "Stop Mode" for additional info.

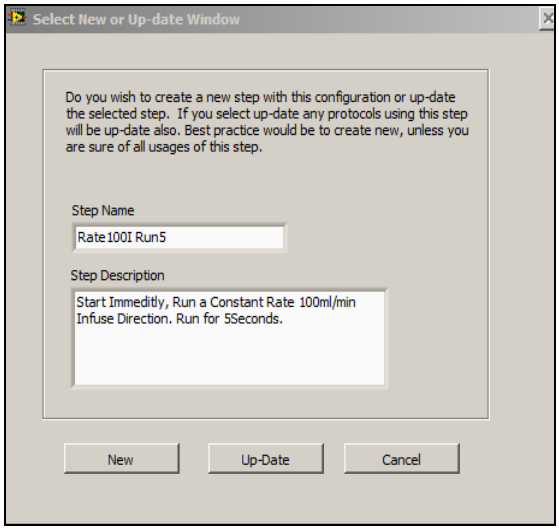
Repeat Mode
See "Repeat Mode" for additional info.

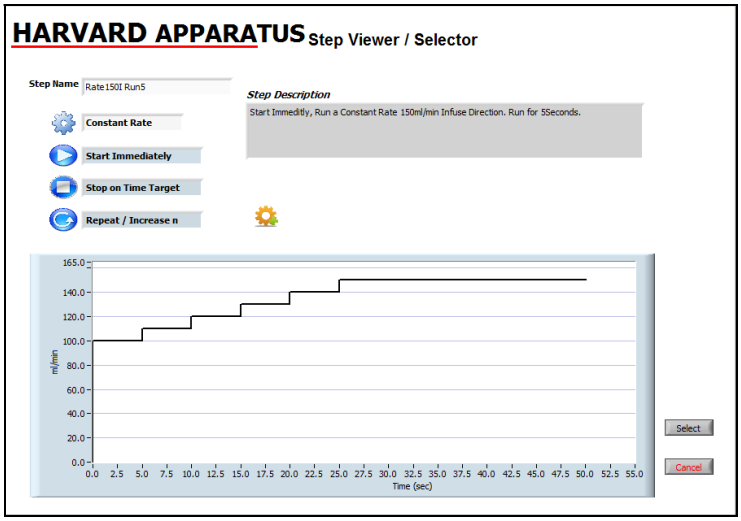
Pump Selector
Choose from peristaltic, syringe, pulsed blood, or ventilator modes.

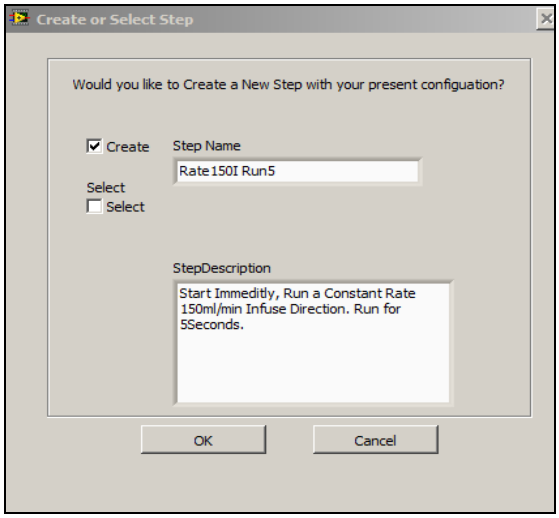
Step Creation/Modification

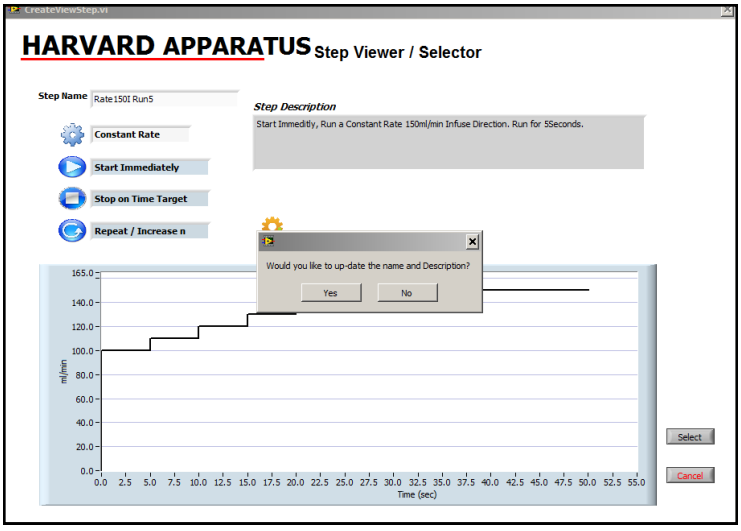
In these windows, you are able to create a new step or update an existing one. The program asks for the user to input a step name and description for each new step or updated step. You can see the step in graphical form using the Step Viewer/Selector.




Select New or Up-date Window
Do you wish to create a new step with this configuration or up-date the selected step. If you select up-date any protocols using this step will be up-date also. Best practice would be to create new, unless you are sure of all usages of this step.
Step Name
Rate100I Run5
Step Description
Start Immediatly, Run a Constant Rate 100ml/min Infuse Direction. Run for 5Seconds.
New
Up-Date
Cancel





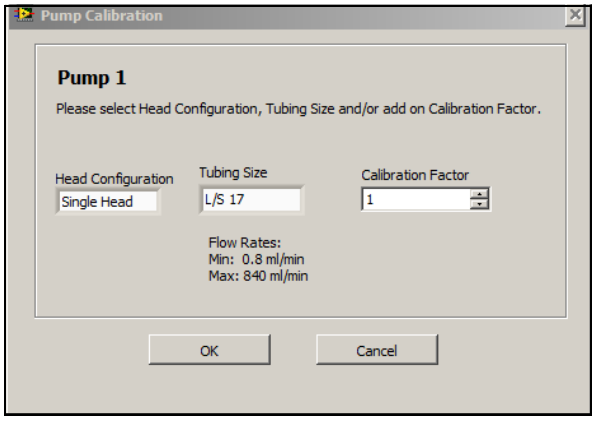


When the step parameters have been selected, a yellow gear with a green cross will pop up.

The user must click this gear and save the step for it to be implemented. Otherwise any changes made will be discarded upon leaving the window.

Pump Calibration

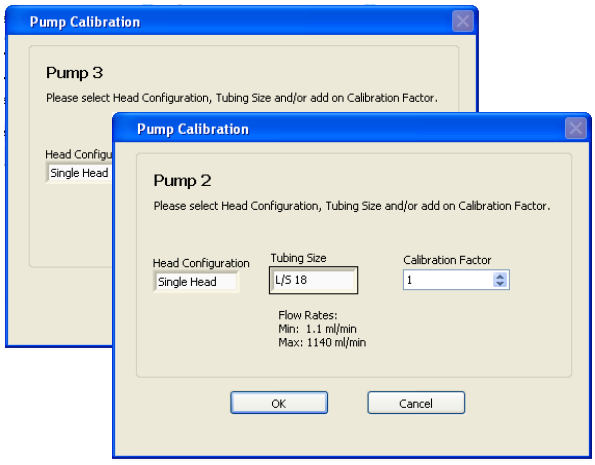
To access the pump calibration menu, click on the image of the pump that you wish to calibrate. From this menu, the head configuration and the correct tubing size can be selected. The calibration factor can also be adjusted.



Head Configuration: Select from single, double, triple, or quadruple head configurations. Note: The max speed of the motor is 300rpm.

Tubing Size: Select the PharMed tubing size that you wish to use. See table below for a description of the available tubing sizes and their corresponding flow constraints. When you have selected a particular tubing size, the minimum and maximum flow rates for the selected tubing will automatically be displayed below.

> **CAUTION:** The user must know the tubing size being utilized for each pump head and verify that the correct tubing size is selected. The pump cannot verify this for the user.



Calibration Factor: The number set for this parameter is the inverse of the variance of the liquid delivered. The calibration factor is selected based on the deviation from optimal flow rate.

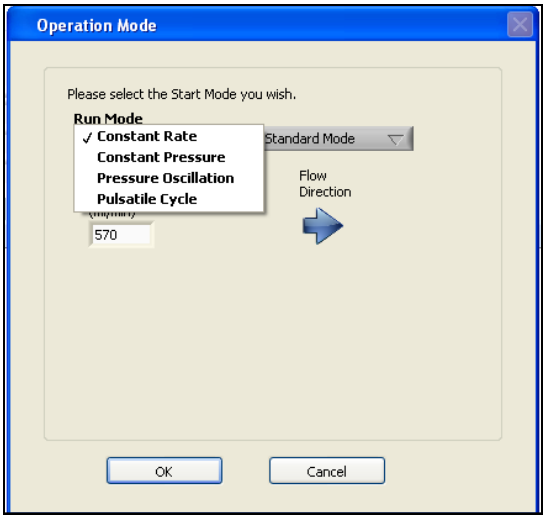
The user may wish to run their perfusate at a specified rate into a graduated cylinder in order to test the actual volume delivered. If the volume delivered is less than the expected amount, the calibration factor can be set at a value greater than one to compensate for this deviation.

* Used for connections—not for use with peristaltic pumps

Specifications for PharMed BPT Pump Tubing							
Tubing Size:	**#13**	**#14**	**#16**	**#25**	**#17**	**#18**	**#82***
ml / min (for single pump head)	0.018 - 18	0.63 - 63	2.4 - 240	5.1 - 510	8.4 - 840	11.4 - 1,140	20.0 - 13000
Inner Diameter in. (mm)	0.03" (0.8)	0.06" (1.6)	0.12" (3.1)	0.19" (4.8)	0.25" (6.4)	0.31" (7.9)	0.5" (12.7)
Barb Size in. (mm)	1/16" (1.6)	1/16" (1.6)	1/8" (3.2)	3/16" (4.8)	1/4" (6.4)	3/8" (9.5)	1/2" (12.7)
Outer Diameter in. (mm)	5/32" (3.9)	3/16" (4.8)	1/4" (6.4)	5/16" (7.9)	3/8" (9.5)	7/16" (11.1)	3/4" (19.1)
Max Pressure-continuous psig (bar)	25 (1.7)	25 (1.7)	25 (1.7)	20 (1.4)	15 (1.0)	10 (0.7)	10 (0.7)
Max Pressure-periodic psig (bar)	40 (2.7)	40 (2.7)	40 (2.7)	35 (2.4)	20 (1.4)	15 (1.0)	15 (1.0)
Use in Single Channel Head	YES	YES	YES	YES	YES	YES	NO
Use in Dual Channel Head	YES	YES	YES	YES	NO	NO	NO
Part Number (25 foot roll) (7.6m)	31101	31102	30340	30339	30338	30337	30552

Operation Mode

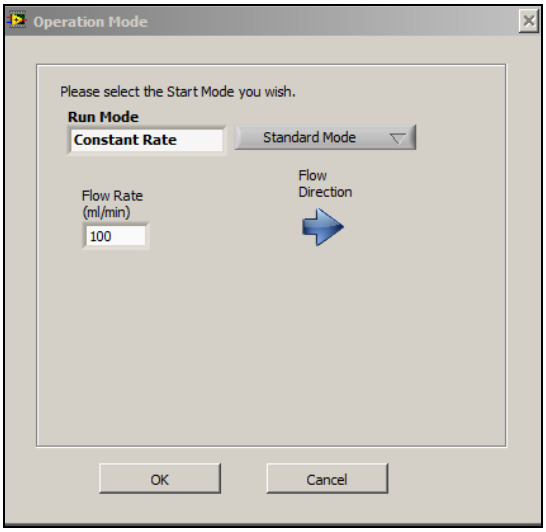
On this screen, Run Mode, Flow Rate, and Flow Direction can be controlled.



Run Mode

There are four options to choose from.

- **Constant Rate**: provides a constant perfusion rate
- **Constant Pressure**: pump alters its flow to maintain constant pressure
- **Pressure Oscillation**: adjusts the flow to bounce between two set pressure points
- **Pulsatile Cycle**: provides a pulsatile flow



In **Constant Rate** mode, flow is set by the user and maintained at a constant rate. Flow direction may be changed.

In **Constant Pressure** mode, constant pressure is set by the user and the pump varies the flow to maintain the pressure setting.

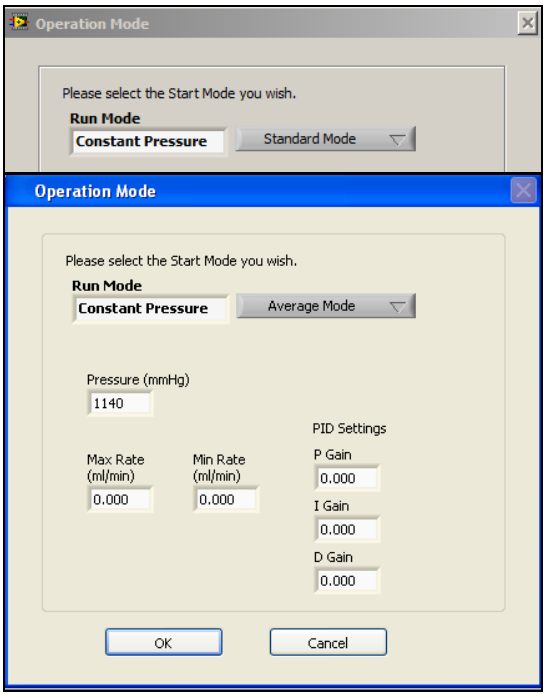
Max rate and min rate determine the maximum/minimum rates at which the pump is allowed to operate.

PID Setting P Gain is the proportional gain which dictates how fast the pump speed is allowed to change in order to reach the set point.

PID Setting I Gain is the integral value used to adjust the baseline and fine tune the rate at which the pump is allowed to move to reach the programmed setting.

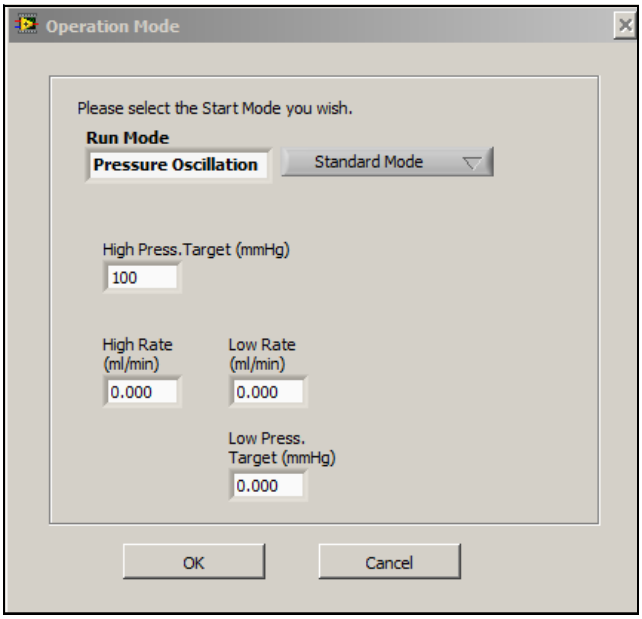
Note: For more information regarding PID controllers, see Appendix B.

Users can also choose between Standard Mode and the Average setting. Standard Mode uses the raw data of each reading. A reading is taken about every 25 nanoseconds. If the signal is very noisy, the Average setting is a better option, which uses a rolling average of the last 40 readings for PID controller feedback.



In **Pressure Oscillation Mode**, the system alternates between using the high rate to obtain the high pressure target and using the low rate to obtain the low pressure target.

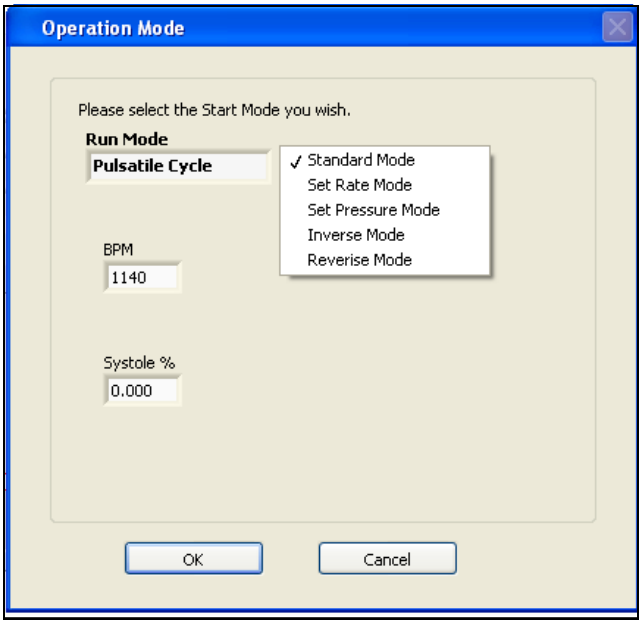
The pressure targets are in units of mmHg and the rates are in ml/min.



In **Pulsatile Cycle Mode**, the program allows for a pulsatile flow to be provided by the peristaltic pumps.

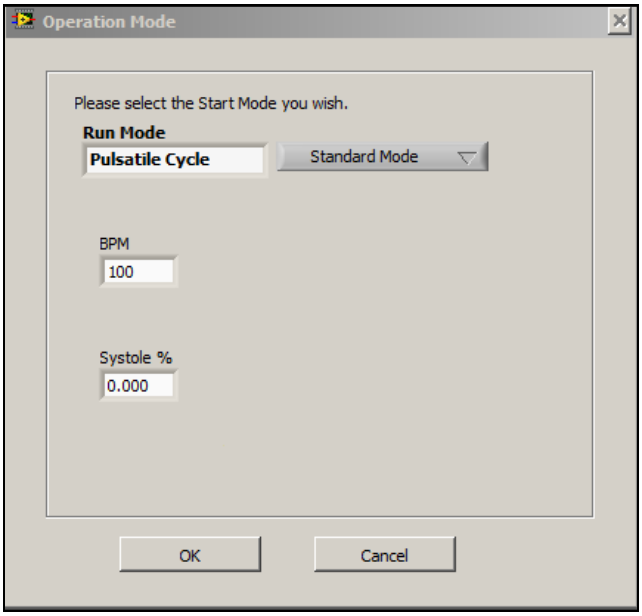
There are five modes of operation:

- -Standard
- -Set Rate mode
- -Set pressure mode
- -Inverse mode
- -Reverse mode



In Standard Mode, the parameter BPM allows the beats per minute to be set.

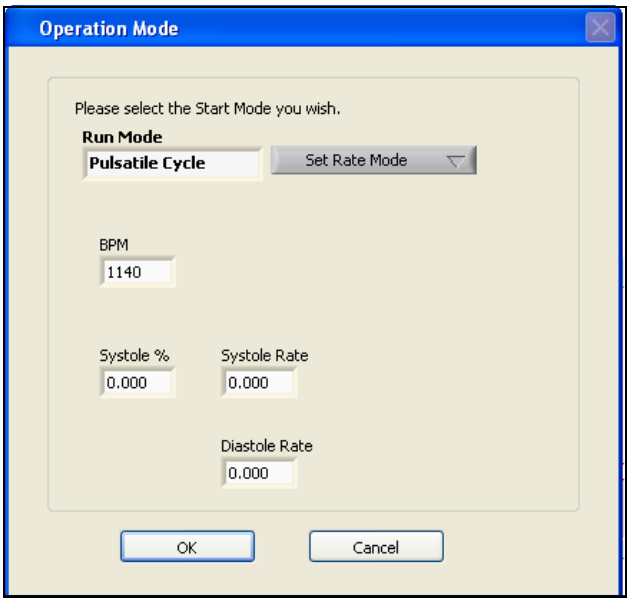
Systole % allows the % of the cycle that is delivering to be set. This value is typically about 35% which must be entered as "0.35".



In Set RATE Mode, Systole % is the same as in Standard Mode.

Systole rate is the rate (in ml/min) that the pump is allowed to increase to reach the set delivery rate.

Diastole rate is the rate (in ml/min) that the pump is allowed to increase to reach the set fill rate.

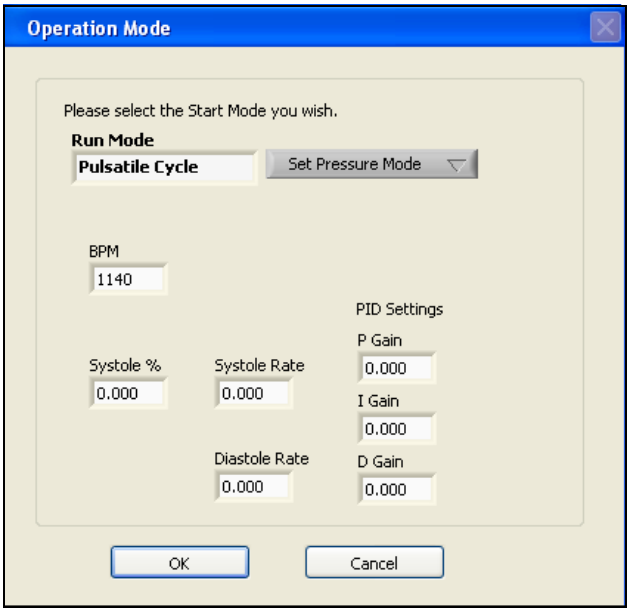


In Set Pressure Mode, constant pressure is set and the pump varies the flow to maintain the pressure setting.

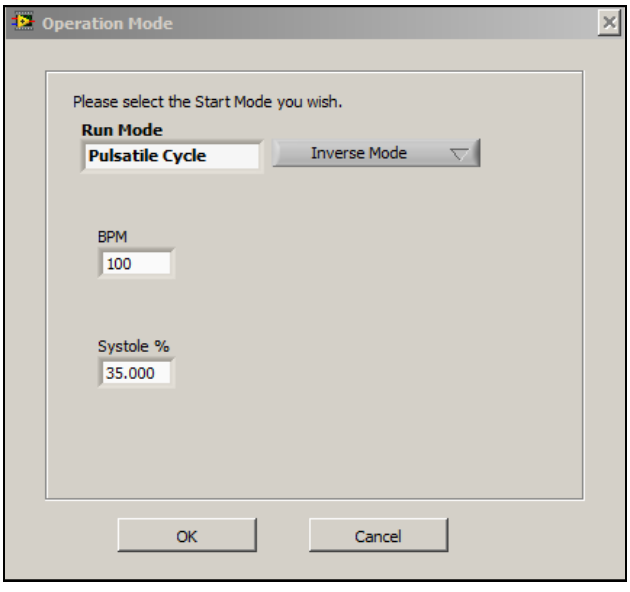
Max rate and min rate determine the maximum/ minimum rates at which the pump is allowed to operate.

PID Setting P Gain is the proportional gain which dictates how fast the pump speed is allowed to change in order to reach the set point.

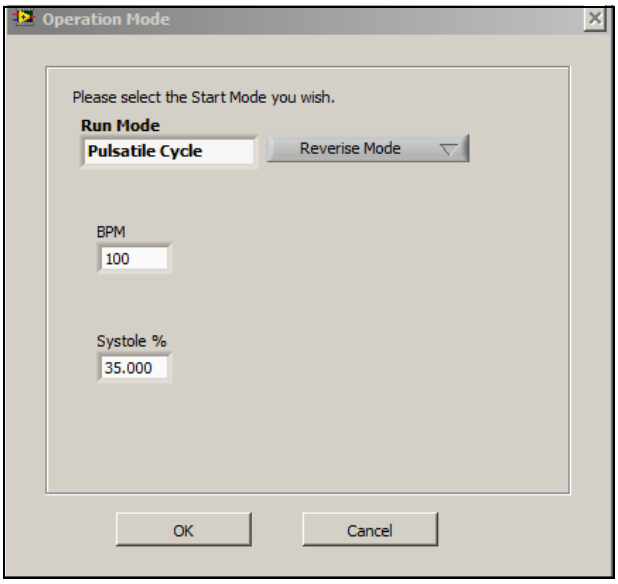
PID Setting I Gain is the integral value used to adjust the baseline and fine tune the rate at which the pump is allowed to move to reach the programmed setting.



In Inverse Mode, the setting for the diastole and systole cycles are reversed. Otherwise, the parameters are the same as in Standard Mode.

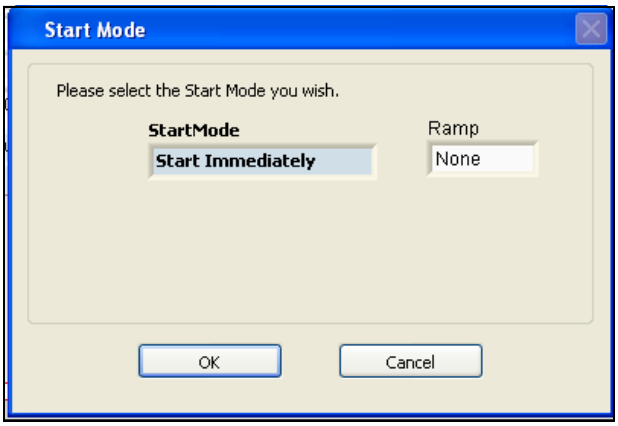


In Reverse Mode, the systole is operated on during the delivery phase. The diastole is operated on during the fill phase.



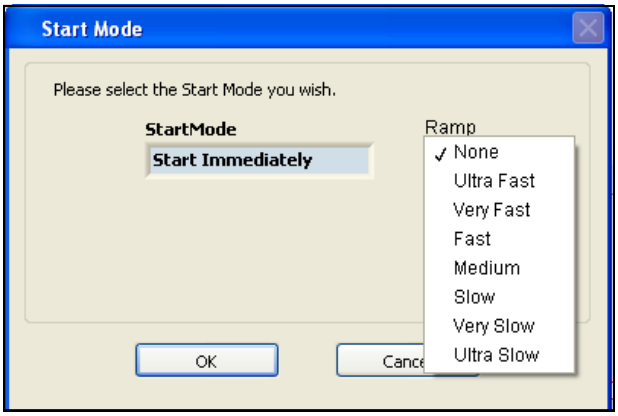
Start Mode

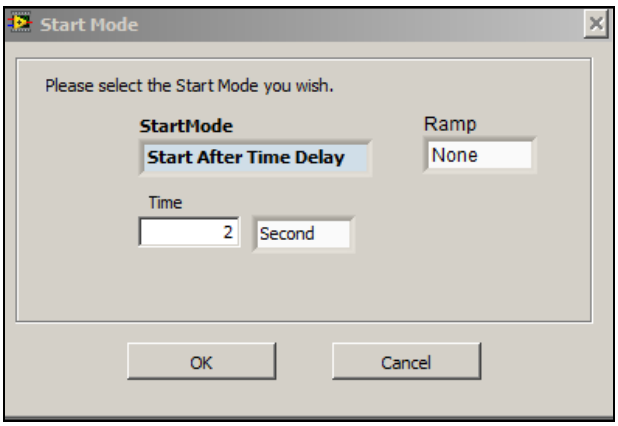
The user has the option to set a time delay before the pump starts running at the rate at which it has been set, or can opt for the "start immediately" setting.



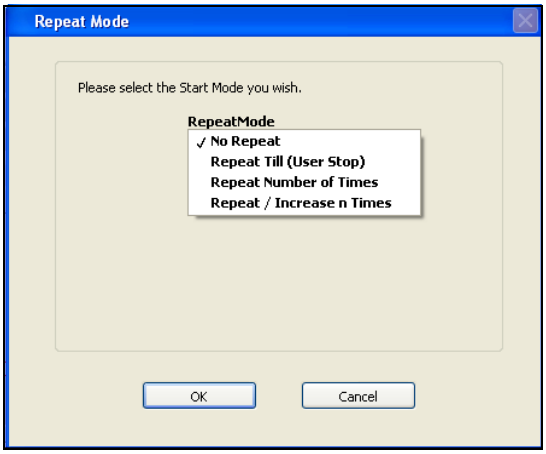
Ramp allows the user to select the speed at which the pump will deliver. There are options in increments ranging from very slow to very fast. "None" is also an option for type of ramping.

Note that the maximum flow rate is a constraint set by the type of tubing used.





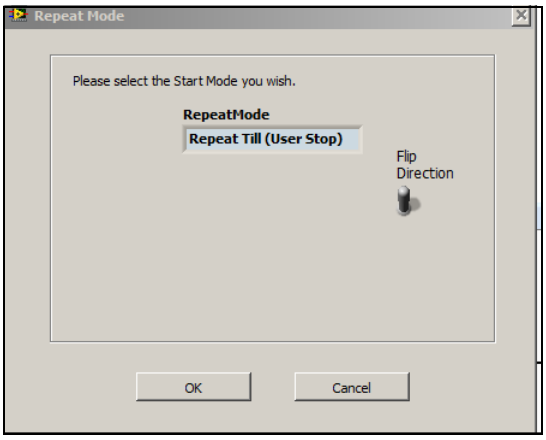
Repeat Mode



Four modes of repeat are allowed:

- No repeat
- Repeat until
- Repeat # of times
- Repeat increase N

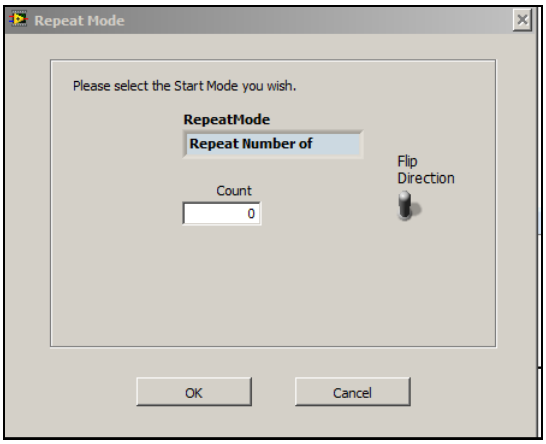
In No Repeat mode, the step does not repeat.



In Repeat Until (User Stop) Mode, the step will repeat until the user stops the program manually.

The Flip Direction switch allows the flow to be reversed.

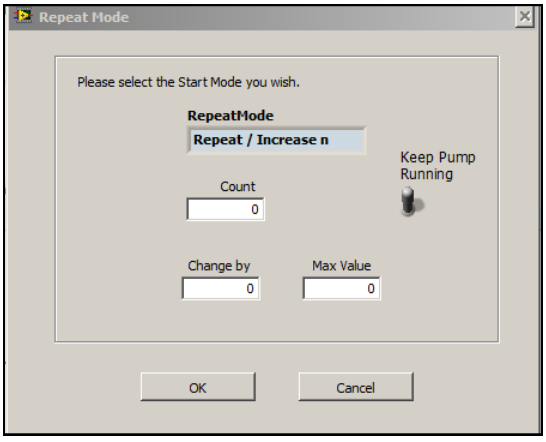
In this mode, the step will be repeated for a fixed number of times.



The Count option allows you to set the number of iterations you want (there is no limit).

The Flip Direction switch up allows the flow to be reversed on every other cycle. 10 repeats with flip direction switch up would result in half of the cycles (5) in the forward direction and half (5) in the reverse direction. Switch down and all cycles in the same direction.

In Repeat/Increase Mode, the speed of the pump increases with every subsequent iteration.



Count allows you to set the number of iterations.

Change by allows you to set how much to increase the speed of the pump for each time the step is repeated

Max value is the maximum flow in ml/min (this is limited by the tubing)

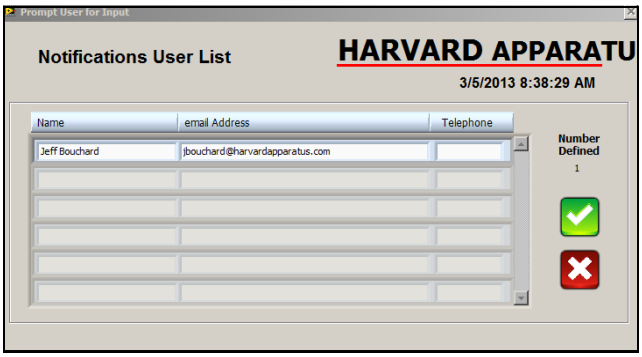
Keep Pump Running allows the pump to continue to run as the final flow rate is reached

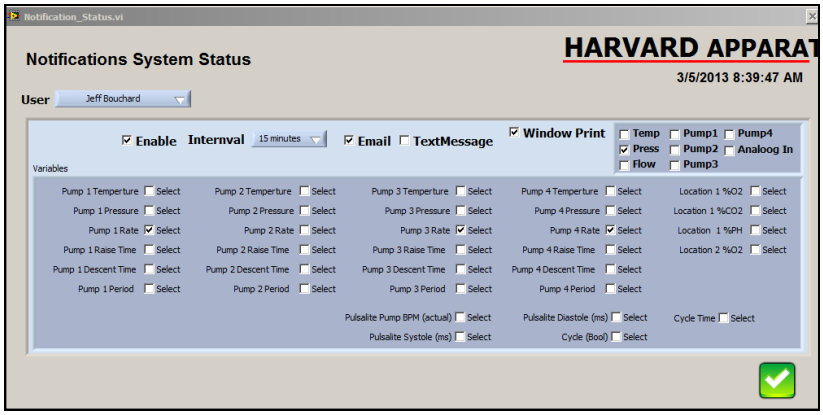
Notifications

This window allows the user to enter contact information in order to be notified by email when certain events occur.

At present, text messaging is not active so entering the phone number has no effect.

This screen allows you to select specific events for which you will receive notification.





Saving Files

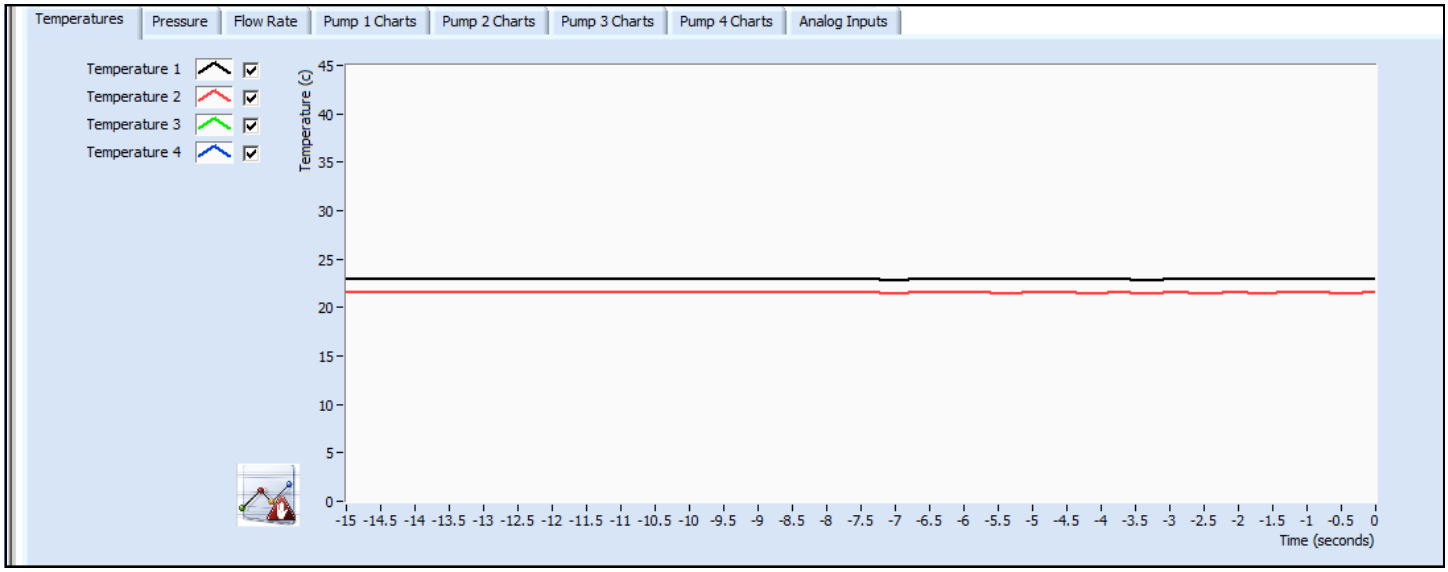
Be certain to create an experiment folder in my documents by naming the experiment (see below "Experiment_Name". Once you save after naming the experiment a folder will be available in the computer's "My Documents" folder and can be copied onto a portable drive and taken to another PC for evaluation.

Chart Readouts

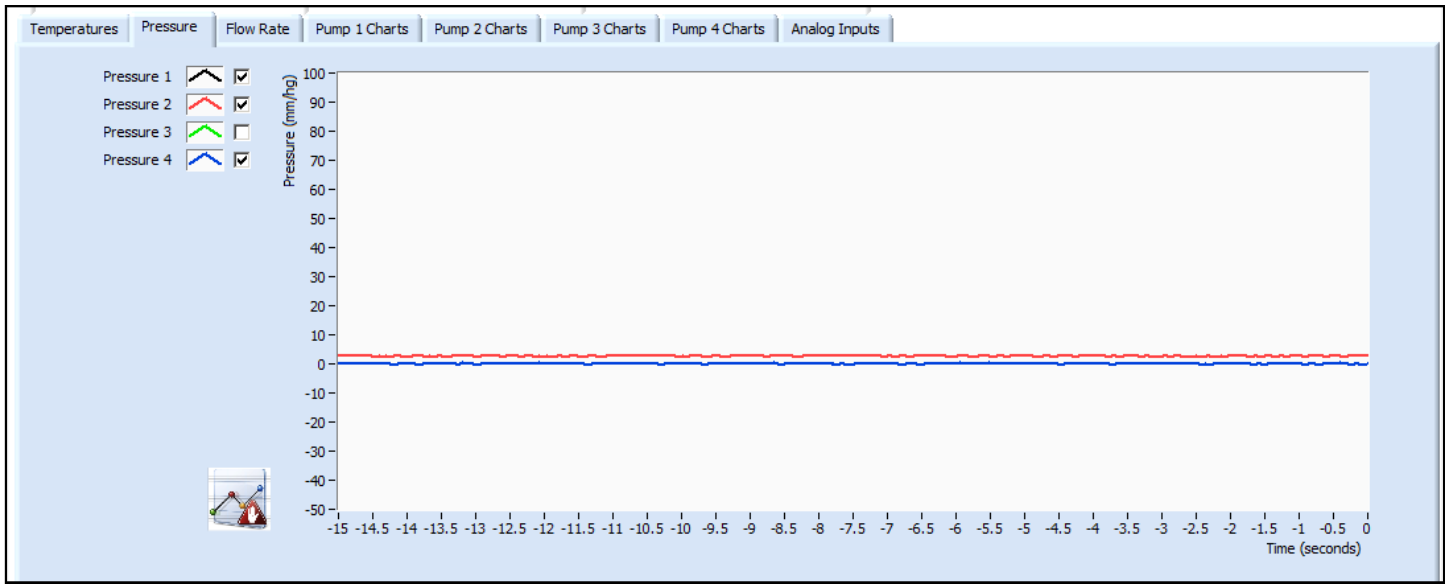
Note: **In any tab, you may click on the top and bottom numerical values on the y-axis at any time to change the max and min values**. Click on the actual high number, it will light up, manually type in the high level you want. Repeat this for the low value of the Y axis. This is done as if you are changing a text value. Also be sure to check the boxes next to the channels that you would like to have plotted.

Analog Inputs

The ORCA controller allows you to feed in any analog signal and this software can capture, display, and save the traces. This is commonly used with the PoleStar gas monitoring system.



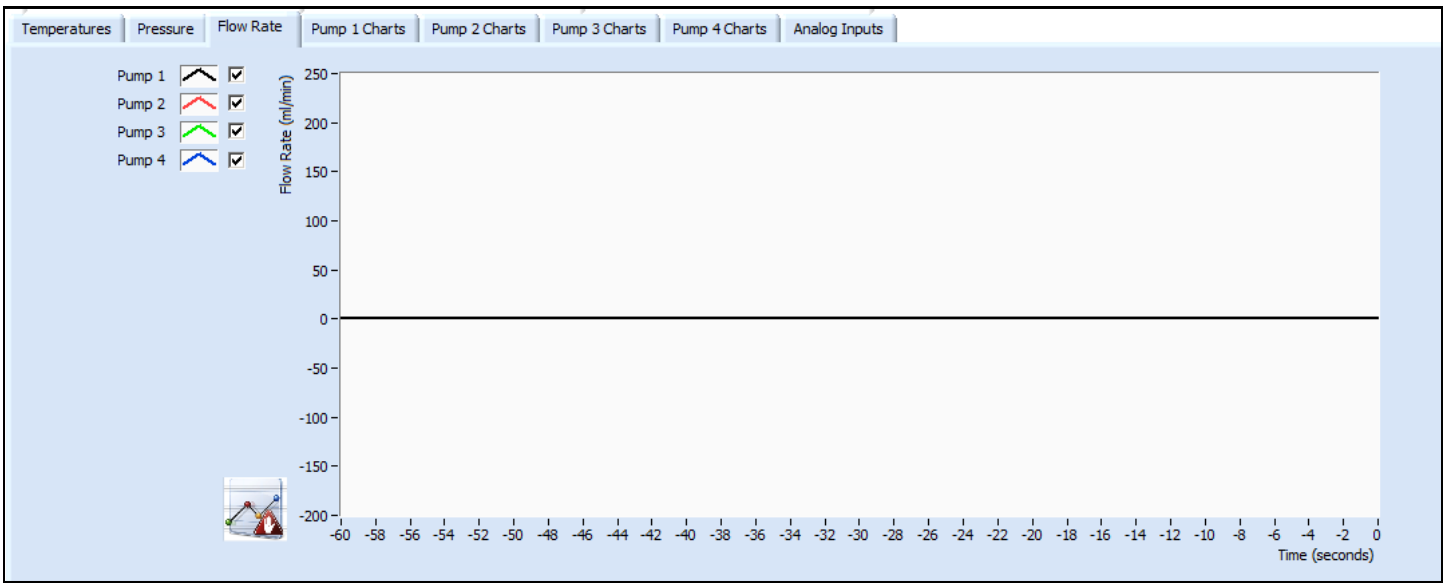
The first tab is for monitoring the temperatures over time of the channels (up to 4) in use.



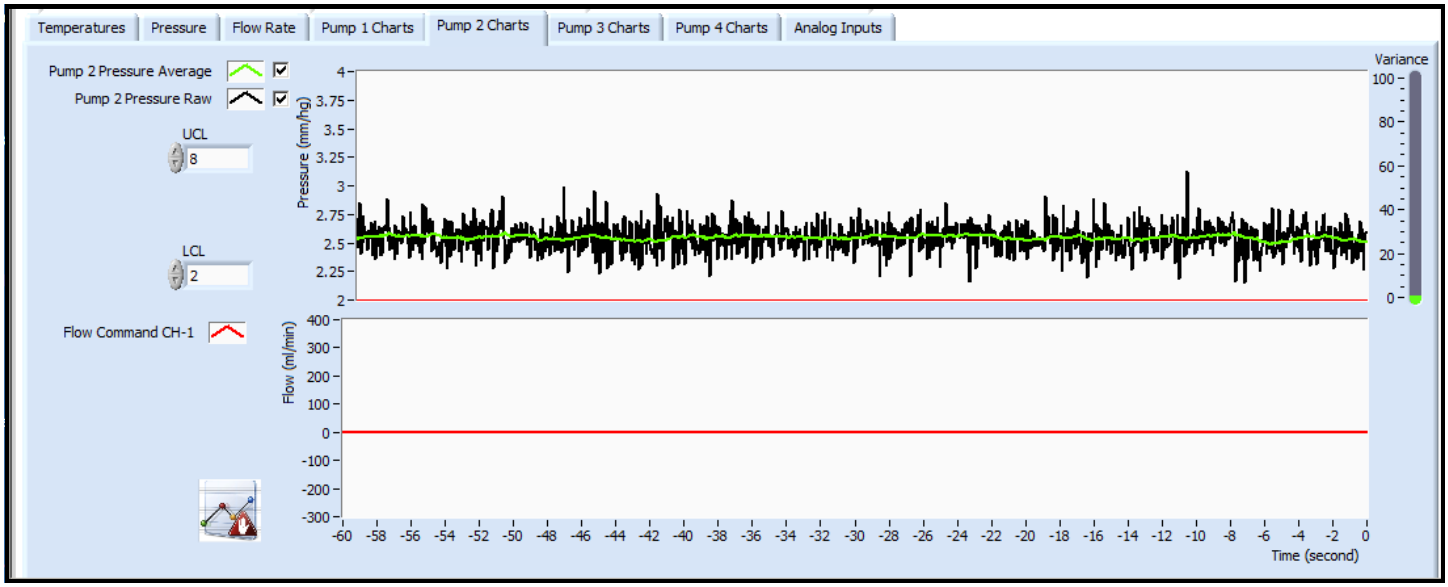
This is an example pressure readout for the various pumps' flow paths.

The pressures corresponding to pumps 1, 2, and 4 are being displayed.

Note: If you do not see all of the traces that you are expecting, one or more of the traces could be overlaid. In order to remedy this, turn off traces sequentially by unchecking the corresponding boxes next to "Pressure 1", "Pressure 2", etc.



The third tab is for monitoring the flow rates over time of the channels (up to four) that you have in use.

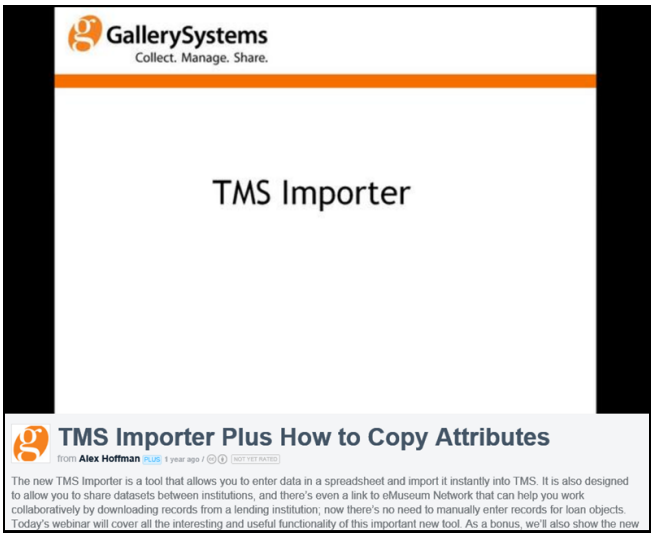


This tab gives the raw and average pressure readings for Pump 2, as well as the flow rate.

Note: UCL and LCL refer to statistical controls that the user develops when a method is validated. They are able to give you a graphic representation so that the user is able to see if there are certain characteristics in the data, for example a "spike", gradual shift, or sine-like wave.

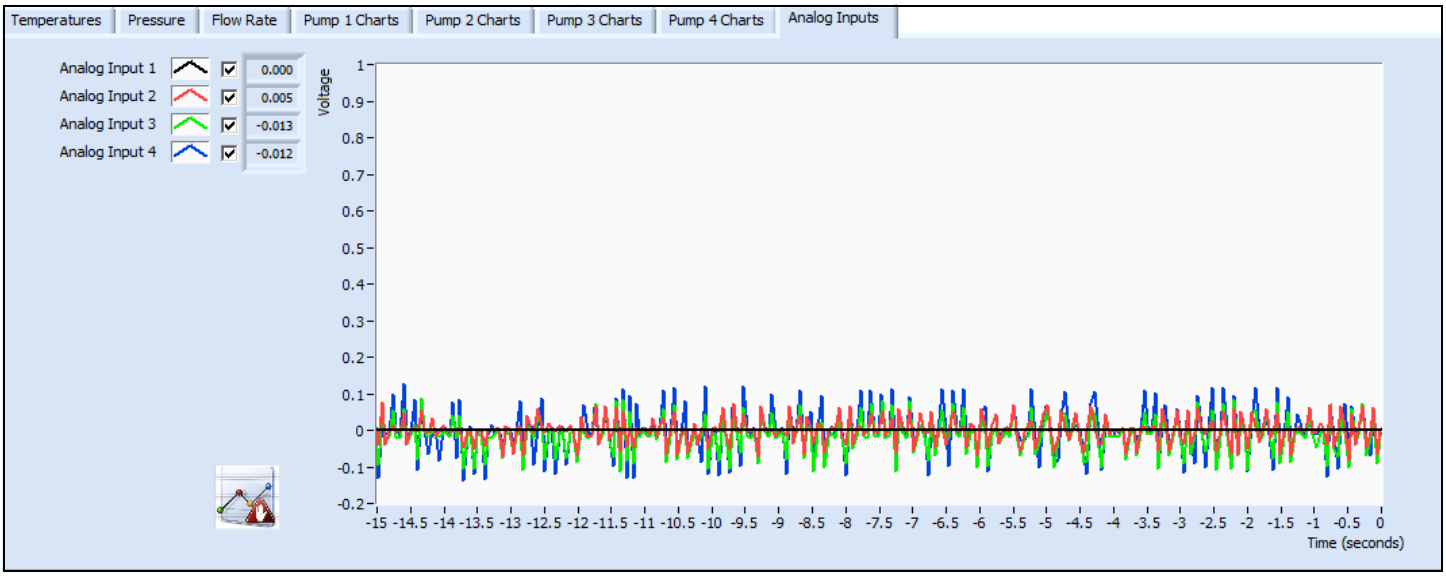
Reading Data Files

In order to read data logged as an Excel file, the computer that you are using to view the files must have a converter program installed. One such program is TMS Importer which can be obtained from http://zone.ni.com/devzone/cda/epd/p/id/2944 or http://vimeo.com/22639555


GallerySystems
Collect. Manage. Share.
TMS Importer
TMS Importer Plus How to Copy Attributes
from Alex Hoffman PLUS 1 year ago / NOT YET RATED
The new TMS Importer is a tool that allows you to enter data in a spreadsheet and import it instantly into TMS. It is also designed to allow you to share datasets between institutions, and there's even a link to eMuseum Network that can help you work collaboratively by downloading records from a lending institution; now there's no need to manually enter records for loan objects. Today's webinar will cover all the interesting and useful functionality of this important new tool. As a bonus, we'll also show the new

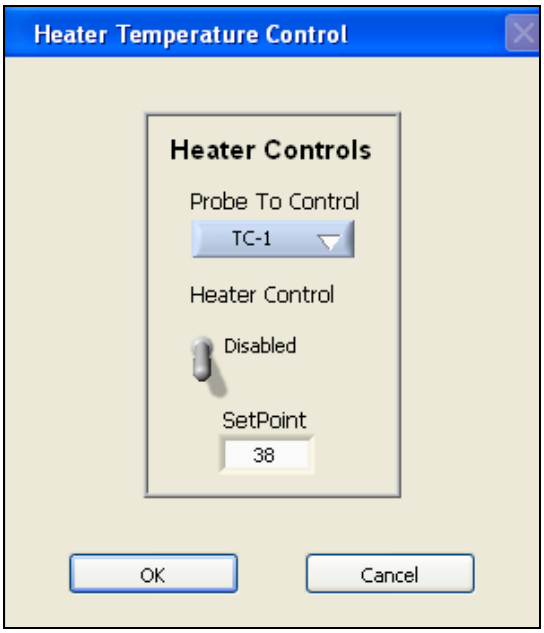
Analog Inputs

The ORCA controller allows you to feed in any analog signal and this software can capture, display, and save the traces.



4.4 Heater Temperature Control & Pressure Channel Calibration

Heater Temperature Control



Access the Temperature Control menu through *Tools—>Heater*.

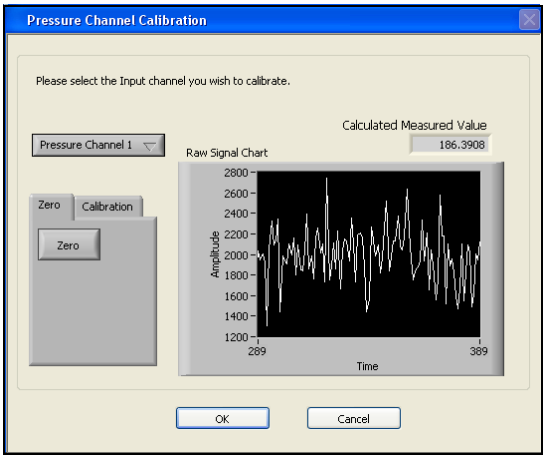
Probe to Control allows the user to select between four different probes, labeled TC-1 to TC-4.

Heater Control can be set to either Enabled or Disabled.

SetPoint allows the user to set the desired temperature in degrees Celsius.

The Pressure Channel Calibration menu can be accessed through *Tools—>Calibration—>Pressure*.

Pressure Channel Calibration



Select the desired pressure channel. You can have up to four channels.

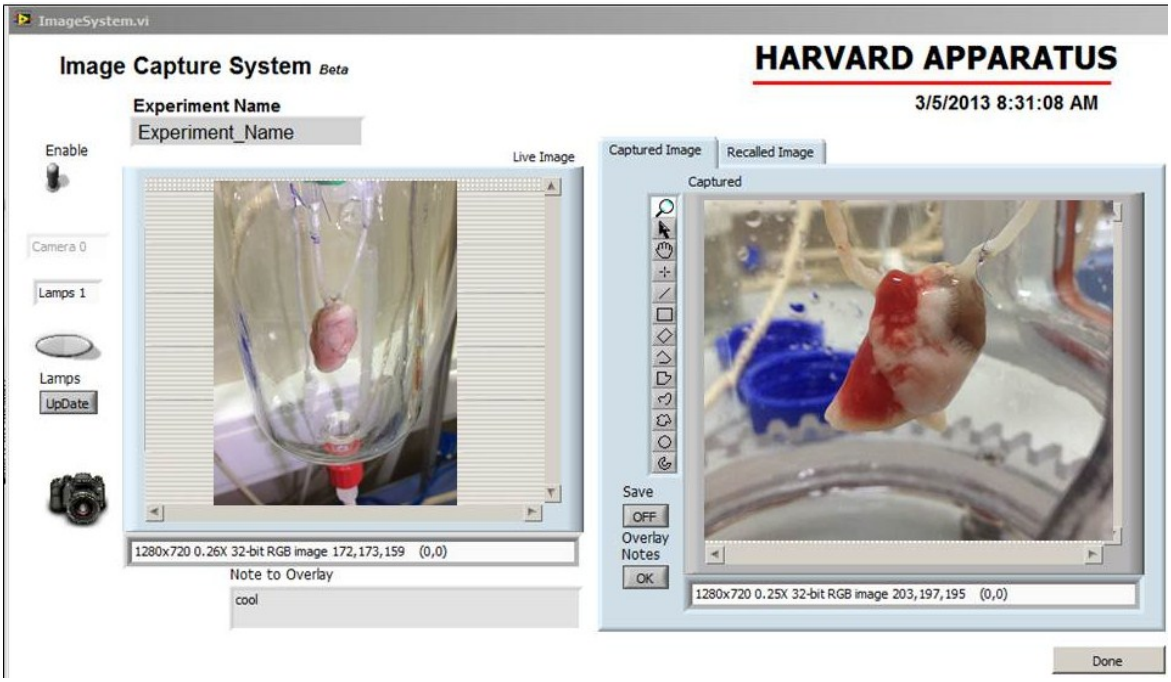
Calculated Measured Value is the instantaneous value for the pressure reading.

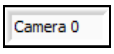
Use the Zero button to zero the actual value reading, and Cal Value along with the Calibration button to calibrate pressure readings. The zero is normally set by opening the transducer to air.

WARNING: You must have a pressure measurement device and a way to increase the pressure. If you press the calibration button and do not have a way to measure the actual pressure you will establish a false reading and not be able to use it.

Manual Pressure Calibrator {31075}

4.5 Image Capture System





Camera allows you to select which camera you want to capture on the screen.

There is no real limit to the number of cameras that you have hooked up. Remember you can always utilize the camera on the laptop as well.

Lamps are used with Dino-lite cameras.
This button turns on and off the lights on the camera.

The camera button lets you take a snap shot and save it on the right screen while still displaying the left screen as live image

- Overlay notes allow you to add notes to the picture before you save it
- Note to overly allows you to add in notes

Note: The Image Capture System is still in beta; some functions may not yet work properly, in which case other software may need to be supplemented.

Chapter 5: Care & Maintenance

5.1 Cleaning

Standard Laboratory protocols may be used. In general we recommend the following steps prior to autoclaving:

A. Flushing with deionized water
B. Washing with 70% IPA
C. Flushing with deionized water
D. Washing with a mild detergent
E. Flushing with deionized water

Stainless steel parts may be sonicated.

CAUTION: Do NOT use bleach as it can cause the chamber to crack and subsequently leak.

Cross-Contamination Prevention, Biohazardous Waste, and Product Disposal

Cross-Contamination Prevention / Universal Precautions

All blood products or products potentially contaminated by blood or other body/animal fluids should be treated as potentially infectious materials. Personal protective equipment should be worn at all times when using the In-Breath Bioreactor to protect personnel from becoming contaminated as well as to help prevent cross-infection and cross-contamination.

Bench tops, equipment, and other potentially contaminated surfaces should be cleaned and disinfected according to the manufacturers' and/or the facility's procedures. Any article used to clean potentially contaminated surfaces should be disposed of as Biohazardous Waste.

CAUTION:

Failure to use the manufacturers' cleaning and disinfecting procedure could result in damage to the surface or equipment.

Biohazardous Waste

Dispose of biohazardous waste according to local Regulatory requirements.

5.2 Replacement Parts List

Part	Part Number
Adapter 1/8” pipe - 1/8 NPT	30325
Barb Plug 1/4” (pk 25)	31111
Barb Plug 3/8” (pk 10)	31112
Barb Plug 1/2” (pk 10)	31113
Barb Plug 3/4” (pk 10)	31114
Blanket Heater Asmbly, 14” chamber	31097
Blanket Heater Asmbly, 10” chamber	31144
Blanket Heater Asmbly, 5.5” chamber	31048
Bubble Trap Ball 14” chamber 1” PolyPro ball	30509
Bubble Trap Ball 10” chamber 3/4” PolyPro ball	30564
Bubble Trap 1/2”	30297
Bubble Trap 1/4”	30450
Bubble Trap 14” head	30295
Bubble Trap 10” head	30442
Bubble Trap O-Ring for 14” chamber	30298
Bubble Trap O-Ring for 10” chamber	30456
Cable ORCA Communication	32007
Cable ORCA Power 110V US	31076
Cable ORCA Power 220V Euro	31077
Cable ORCA Power 220V UK	31179
Cable ORCA Power Australia	0680009101
Cable ORCA Power Ireland	0680009201
Cable ORCA Power Denmark	0680009301
Cable ORCA Power India	0680009501
Cable ORCA Power Israel	0680009601
Cable ORCA Power Italy	0680009701
Cable ORCA Power Switzerland	0680009801
Cable, 5ft F-M 9 Pin, Pump to Controller	31072
Cap, Male Luer Lock, Kynar, pk 25	31086
Cannula Adapter Kit for Rodent Lung	31116
Cannula Adapter Kit for Rodent Heart	31117
Chamber 14” Asmbly	30240
Chamber 10” Asmbly	30260
Chamber 5.5” Asmbly	30320
Chamber Window & Gasket 14”	31085
Chamber Window & Gasket 10”	31084
Chamber Window & Gasket 5.5”	31083
Clamp, 1/8” (3.18mm), pk 25	31087
Clamp, 1/4” (6.35mm), pk 25	31088
Clamp, 3/8” (9.53mm), pk 25	31089
Clamp, 1/2” (12.7mm), pk 25	31091
Connector, 1/8” (3.18mm), pk 10	31092
Connector, 1/4” (6.35mm), pk 10	31093
Connector, 3/8” (9.53mm), pk 10	31094
Connector, 1/2” (12.7mm), pk 10	31095
Quick Connector (Chamber Fill & Drain 3/8”)	31211
Quick Connector (Chamber Fill & Drain 1/4”)	31210
Connector 1/4”Barb—1/2” NPT	30532
Connector 1/8”Barb—1/4-28” UNF pk 25	30407
Connector 1/4”Barb—1/8” NPT pk 10	30401
Elevator Assembly, 14” Chamber	30753
Elevator Assembly, 10” Chamber	30785
Fitting, Horizontal Chamber Drain Level (25ea)	880484
Fitting Luer Lok x 1/16 NPT (25ea)	31180
Fitting Right Angle (14” Chamber) (25ea)	30420
Fitting Luer Female 1/4—28 (25ea)	31163
Fitting, 1/4” Polycarb Straight w/ Luer Port (25ea)	30904
Fitting, 3/8” Polycarb Straight w/ Luer Port (25ea)	30899
Fitting, 1/2” Polycarb Straight w/ Luer Port (25ea)	30905
Fitting Tee (25 ea)	31138
Fitting Tee (25 ea) slip Luer	512294
Heater Reservoir (115V)	30250
Heater Reservoir (220V)	30247
Liner for Heater (25 each)	31096
Manual, ORCA	30752
Nut Luer Clear (25 each)	31141
Nut Luer Green (25 each)	31142
Nut Luer Red (25 each)	31143
Nut Thumb for Chambers (5 each)	31145
PID Temperature Controller	30875
Pipe Barb 1/4” - 1/4” NPT (25 each)	31133
Port, Swabable, MLL, pk 25	31081
Port Extra Chamber 14” chamber	30410
Port Extra Chamber 10” chamber	30470
Pressure Calibration Tool	31075
Probe, Flexible Implantable	31080
Probe Temp Sensor (12” 30.5 cm x 1/8”OD)	31079
Probe Temp Sensor (12” 30.5 cm x 1/16”OD)	30960
Probe Temp Sensor (6” 15.24 cm x 1/8”OD)	31078
Probe Temp Sensor (6” 15.24 cm x 1/16”OD)	30959
Probe Temperature, Extension Cable	30961
Pump peristaltic single head single channel	31061
Pump peristaltic dual head single channel	31062
Pump peristaltic four heads single channel	31063
Pump peristaltic single head dual channel	31064
Pump peristaltic dual head dual channel	31065
Pump peristaltic single and dual channel head	31066
Reservoir Bottle w/ Lg Animal Fittings 4,000 mL	31099
Reservoir Bottle w/ Sm Animal Fittings 500 mL	31105
Reservoir Bottle w/ Sm Animal Fittings 1,000 mL	31104
Reservoir Bottle w/ Sm Animal Fittings 2,000 mL	31100
Screw Mounting Peristaltic Pump for 2 heads	31067
Screw Mounting Peristaltic Pump for 3 heads	31068
Screw Mounting Peristaltic Pump for 4 heads	31069

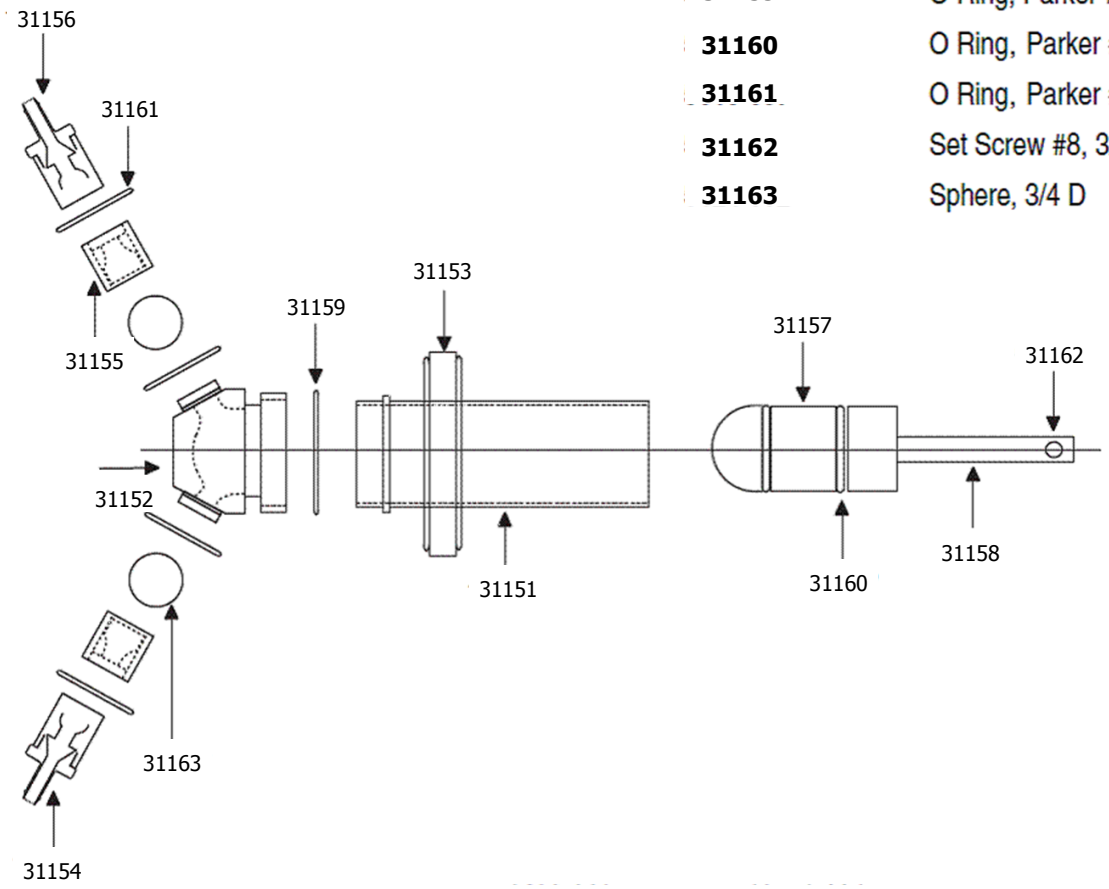
Parts List (cont.)

Spring, for 14” Chamber Heater	31209
Spring, for 10” Chamber Heater (2ea)	31207
Spring for 5.5” Chamber Heater (2 long & short)	31208
Transducer Pressure (Pendotek 10ea)	31074
Transducer Pressure Cable	31073
Tubing Connection Kit, Large Animal	30695
Tubing Connection Kit, Small Animal	30690
Tubing PharMed (1/32”, 0.8.mm ID), 25ft	31101
Tubing PharMed (1/16”, 1.6mm ID), 25ft	31102
Tubing PharMed (1/8”, 3.1mm ID), 25ft	30340
Tubing PharMed (3/16”, 4.8mm ID), 25ft	30339
Tubing PharMed (1/4”, 6.4mm ID), 25ft	30338
Tubing PharMed (5/16”, 7.9mm ID), 25ft	30337
Tubing PharMed (3/8”, 9.5mm ID), 25ft	31103
Tubing PharMed (1/2”, 12.7mm ID), 25ft	30552
Tubing PharMed (3/4”, 19.1mm ID), 5ft	30553
Tubing Teflon 1/8” (3.18mm) OD, 1ft	30358
Tubing Teflon 1/4” (6.35mm) OD, 1ft	30345
Tubing Teflon 3/8” (9.53mm) OD, 1ft	30346
Tubing Teflon 1/2” (12.7mm) OD, 1ft	30347
Tubing Tygon 1/8” (3.18mm) ID	30341
Tubing Tygon 1/4” (6.35mm) ID	30342
Tubing Tygon 3/8” (9.53mm) ID	30343
Tubing Tygon 1/2” (12.7mm) ID	30344
Y Barb Fitting 3/16” (4.77mm) (10ea)	31124
Y Barb Fitting 1/4” (6.4mm) (10ea)	31125
Y Barb Fitting 3/8” (9.53mm) (5ea)	31126

Pulsatile Pump grease {31164}

Part No.	Replacement Part
31151	Cylinder
31152	Head–Valve
31153	Nut–Coupling
31154	Nozzle–Suction
31155	Body–Valve
31156	Nozzle–Discharge
31157	Piston
31158	Shaft–Connecting
31159	O Ring, Parker #2-140
31160	O Ring, Parker #2-133
31161	O Ring, Parker #2-24
31162	Set Screw #8, 32 x 3/16
31163	Sphere, 3/4 D

Pulsatile Pump Head {31150}



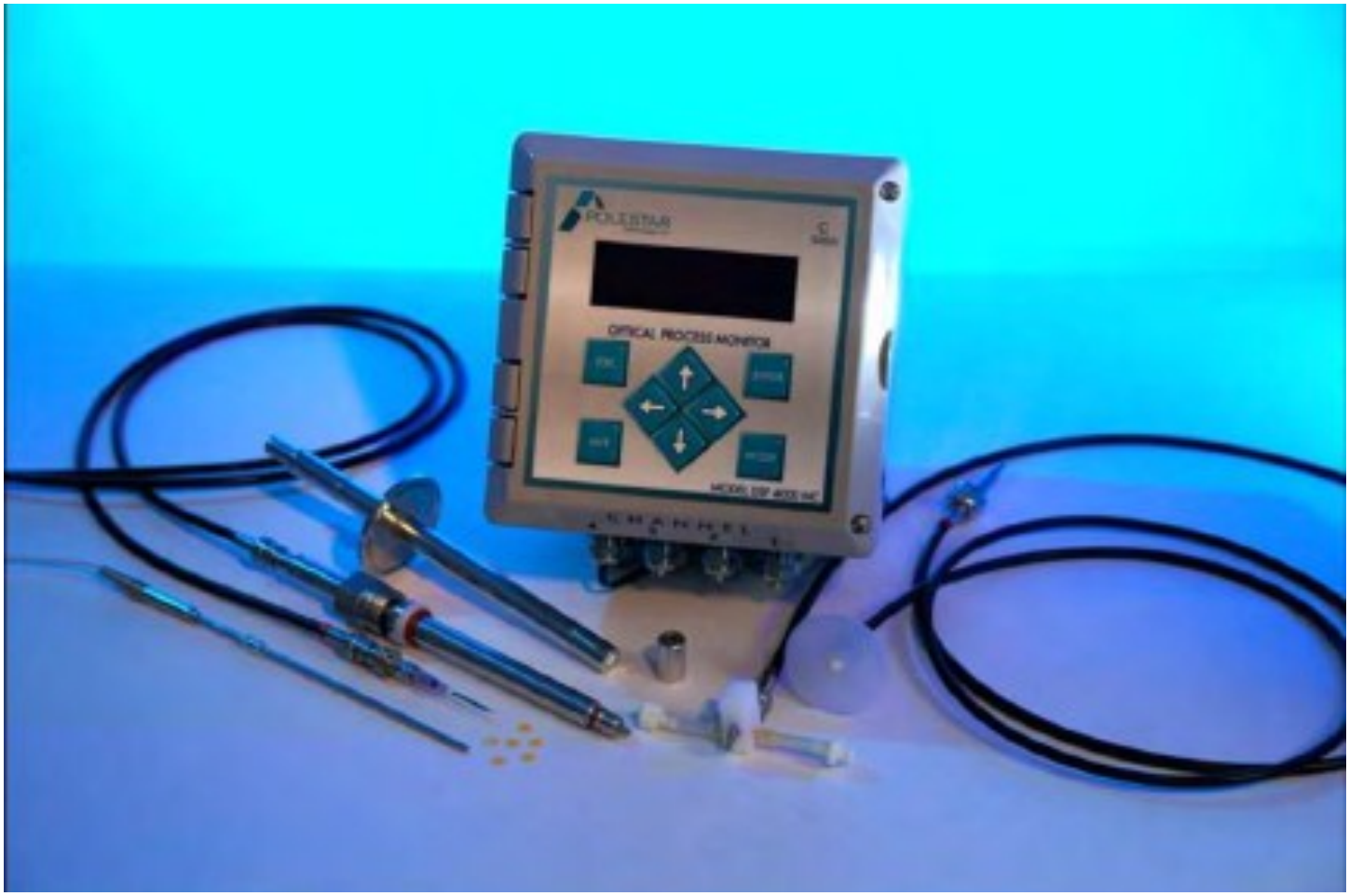
Appendix A: PoleStar Setup and Operation

Application Instructions

Polestar Fluorescence-based Optical Biosensors

Harvard Apparatus

Version 1.21 061112



Written primarily for applications involving isolated organ and tissue.
See manufacturer's manual for details regarding use in standard applications.

This guide is NOT a replacement for the manufacturer's manual.

Table Of Contents

1 Introduction, Manufacturer's details

2 Safety Note

3 General Description, Application

- **3.1 Measurement Specifications**

4 Initial Setup of DSP4000 Unit/Quick-start Guide

- **4.1 Installing the USB onto Computer**
- **4.2 Connecting RTD Probes to Sensors**
- **4.3 Integrating Sensors into System Setup**
- **4.4 Connecting DSP4000 to Data Acquisition System**

5 Calibrating Sensors, General

- **5.1 Set Barometric Pressure**
- **5.2 Units Conversion, Data Acquisition 2-Point Calibration**
- **5.3 Oxygen Sensor Calibration**
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6 Viewing Data on DSP4000

- **6.1 Channel Readings – O2, CO2, pH Parameters**
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- **7.1 Updating Lot Number for New Sensor Calibration**

8 Cleaning and Maintenance

9 Troubleshooting Guide/ Ordering Information

1 Introduction, Supplier's detail

These Operating Instructions describe the function and use of the DSP4000 Optical Process Monitor. This manual is to be considered part of the system for its proper use and operation and should be kept close to the system at all times. The information provided on the DSP4000 has to be read in conjunction with the remaining system documentation for the application to which it is being put to use.

All the information contained in these instructions has been drawn up after careful examination but does not represent a warranty of product properties. Alterations in line with technical progress are reserved.

This DSP4000 unit is manufactured by:
POLESTAR TECHNOLOGIES and supplied to you by HARVARD APPARATUS. All inquiries regarding this system addition/application should be directed to Harvard Apparatus or to the Harvard Apparatus Authorized Distributor from whom this unit was purchased.

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2 Safety Note

3 General Description, Application

Warning:
☞ the equipment is designed for use in general laboratories, light industrial and office environments. Operation in hazardous areas and/or in a flammable atmosphere is not permitted.

The DSP4000 Optical Process Monitor can be configured for 1, 2, 3, or 4 Channels (defined by the number of LED modules present in the system). Units are identical in functionality

though the total sensor capacity may differ. In the descriptions below all configurations will be referred to as the "DSP4000."

The DSP4000 is an Optical Process Monitor designed to emit and sense light in a specific range of wavelengths for the purpose of determining the concentration of the presence of the parameter(s) for which the connected sensor(s) is (are) configured (pH, O_2, CO_2).

The sensors are supplied in a range of configurations to meet the various application specifications of the end users. Most frequently researchers who purchase the system from Harvard Apparatus will be utilizing flow-through sensors for use in one of the many perfusion systems available; however, in vivo applications are also common in which a puncture probe may be used. General purpose probes can also be used and immersed in any fluid. O2 and CO2 sensors can operate with fluid or with gas and therefore a wide variety of applications is possible.

The DSP4000 can be configured for 1, 2, 3, or 4 channels for one, two or all three of the parameters currently available (pH, O_2, CO_2). A dual channel system can be configured for measurement of 2 of the same parameters (requiring only one purchase of the firmware for that measurement) or 2 different parameters (as long as the firmware for the parameters of interest are both purchased) or 3 different parameters, with only 2 of the 3 sensors connected at any one time. This manual supplement is primarily designed to guide researchers utilizing the system for isolated perfused organ and tissue applications or who will be calibrating their sensors in the ranges outlined in section 3.1 of this document.

3.1 Measurement Specifications

Oxygen

Measurement ranges available:

High: 0-500% Air Sat

Low: 0-20% Air Sat

Units of Measurement available:

O2 %

O2 % Air Sat

O2 PPM (or PPB, or SUB-PPB)

O2 mmHg

O2 deg

pH

Measurement ranges available:

Low Physiologic: 4 - 7

Mid Physiologic: 5.5 – 10

Carbon Dioxide

Measurement range:

0 – 30 %

Units of measurement available:

CO2 %

CO2 mmHg

SMR

4 Initial Setup of DSP4000 Unit/Quick-start Guide

Please refer to the Quick Start Guide that was supplied with your DSP4000 unit. It is especially important to do the following upon initial setup:

* Open the door to the unit (loosen screws with fingers or screwdriver) and do the following:

    - Remove the USB Key and plug it into your PC and copy the contents to your Desktop (or preferred location on your computer). Take special note of the manual (recommended to print a copy if desired). Return the USB Key to the Unit

    - Verify that the toggle switch (next to USB KEY) is in the "OFF" Position before plugging in the AC Power Adapter (See Figure 2-3 of DSP4000 Optical Process Monitor User's Manual)

* Turn "ON" the DSP Monitor by flipping the toggle switch. The Monitor should warm up for 30 minutes before first time use and at any time when powering on from a full shut down (where the toggle switch inside the unit is in the "off" position). Generally the unit can be left in Standby Mode (the Main Menu is displayed) between uses unless one of the following requires a full shut down:

    • You are connecting I/O as described in Section 4 ("Electrical Connections") in the DSP4000 User's Guide

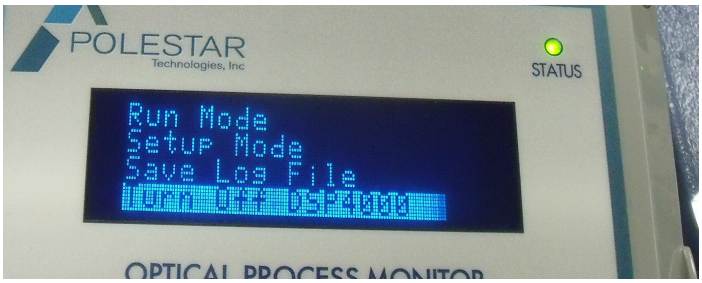
    • You need to upgrade the operating system to a new version. The DSP4000 will prompt you to upgrade the software when you restart the system (see Figure 2-12)

    • There will be an extended period between uses

* Refer to Quick Start Guide for connection of Fiber Optic Cables. NOTE: You must align the small tab on the ST Connector on your sensor with the notch in the Fiber Optic cable or the fitting will not connect.

* Recommended not to re-screw lid until your setup is complete and the unit is fully calibrated and ready to use

* **NEVER use a Power Strip to power on and off your DSP Unit**. This results in a "fuzzy" power cutoff that interferes with the memory and proper functioning of the unit.



Proper methods for powering down the DSP4000:

- Go to Main Menu and select "Turn Off DSP4000." This allows you to put the unit into standby and avoid having to wait for a 60 minute warm-up period at the next use

- If you wish for a full power shutoff, then after the above step, open the DSP4000 and flip the toggle switch to "OFF."

Note: Saving the Log file is optional. If you have performed a calibration procedure or changed any other setting, or recorded measurement data onto the log file, it is advisable to save the log file.

4.1 USB Key

* It is important to identify which sensor was assigned to each channel in order to match the sensor/fiber optic cable and channel with the correct parameter, units and calibration data that were used during testing. Instructions on how to do this are described in section 2.4 of the DSP4000 Optical Process Monitor User's Manual. You can change the assignments later by loading each sensor's calibration file to a different channel, as discussed in Chapter 3, "Managing Configuration Files."

* Removing the USB Key while unit remains under power (without powering off the DSP4000): For full instructions please refer to DSP4000 Optical Process Monitor User's Manual section 2.8.

This is done when you wish to review the log file or update lot #/calibration information (such as when a new sensor is purchased). Using this method also closes the current log file such that when measurement resumes, you will be recording onto a new log file. **note this does not work with older USB Keys where the software may freeze

4.2 Connecting RTD (Temperature) Probes to DSP4000 and Sensors

If your RTD Probe(s) were not purchased with the system and are purchased as an add-on, they will need to be wired into the DSP4000. (Additional information can be found in Section 4.2 of the DSP4000 Optical Process Monitor User's Manual.)

Step 1: Remove the three-pronged connector from the end of the RTD Probe (using a very small screwdriver) and carefully unwind the bare wire terminations and straighten them out

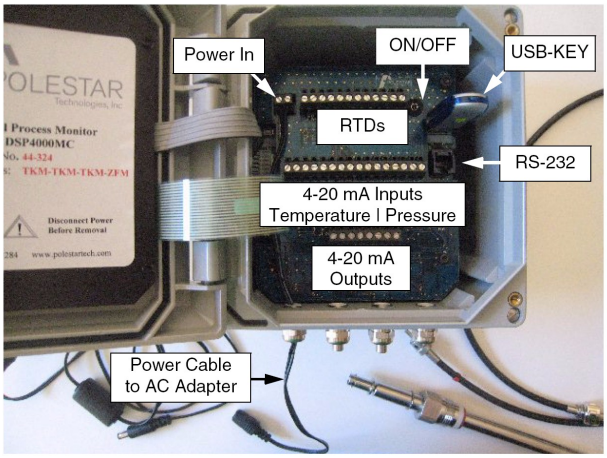
Step 2: Power off the DSP4000 unit by turning it off in the menu and then with the toggle switch inside the case. Unplug the unit from the AC Adapter.

Step 3: Using a small flathead screwdriver loosen the metal clamps for the applicable RTD inputs.

Step 4: The four cord grips on the bottom panel enable you to bring connecting wires into the DSP4000 enclosure while maintaining a water-tight seal. To bring the wire into the DSP4000 enclosure:

- Loosen the locking nut on the cord grip and remove the cylindrical metal plug. Figure 4-2 shows a DSP4000 where the left-most and right-most cord grips have already been used for the power cable and an RS-232 connection, respectively. The plug has been removed from the grip next to the RS-232 cable.

Channel	Terminal Labels		
Channel 1	RTD1_Black [1]	RTD1_Red [2]	RTD1_White [3]
Channel 2	RTD2_Black [4]	RTD2_Red [5]	RTD2_White [6]
Channel 3	RTD3_Black [7]	RTD3_Red [8]	RTD3_White [9]
Channel 4	RTD4_Black [10]	RTD4_Red [11]	RTD4_White [12]



- Store the cylindrical plug in a safe place.

Step 5: Insert the bare wires as per the above table. Clamp down using screwdriver. (NOTE: The white wire is now black—the black are interchangeable)

Connect the threaded RTD probe tip into your sensor. The Flowcells and the Tri-fold Flowcells

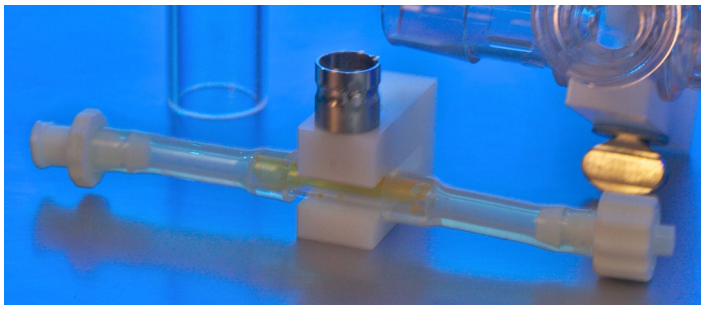
both have built-in threaded ports to accommodate the RTD probe.

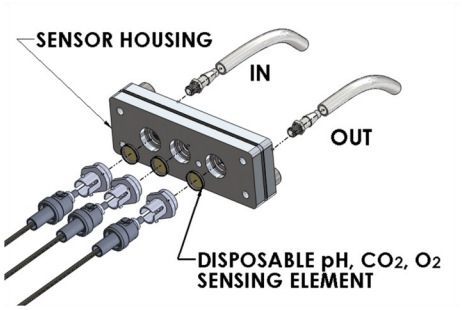
4.3 Integrating Sensors into System Setup

The Flow cells in the cell mounts have male and female luer terminations.

Make sure that you always use a non-gas permeable or low gas-permeable tubing such as Tygon R-3603 or Tygon S-50-HL. Using a Luer-Barb connector kit, you can fit multiple barb sizes to the luer terminations or connect these to each other end-to-end (for multiple sensor types).

The Tri-fold Flowcell has four threaded holes set in a square configuration (0.20" deep, 10-32 Thread/M5 Thread) to accommodate multiple mounting options. It can also be placed on the lab bench or clamped using rod/clamp system to the relevant portion of the perfusion system.





4.4 Connecting DSP4000 to Data Acquisition System

• If you purchased a DAQ-DSP4000 Interface Cable, this has come preinstalled and configured to work with a standard 0-10V Analog to Digital Data Acquisition System (such as a PowerLab, Dataq, NI-DAQ, iWorx, Biopac, or other). These cables terminate in a Male BNC connector. Utilizing DAQ hardware that has analog inputs other than female BNC requires the use of an adapter to your input type or removal of the BNC connector to bare leads.

• Connect the BNC output cables to the Analog Input BNC connectors on your Data Acquisition System. Please connect to the channel on which you wish the particular reading to be recorded.

Sensor Parameter	Units	4 mA =	20 mA =
O_2 High Level	mmHg	0 mmHg	5000 mmHg
	%	0%	100%
	% Air Sat	0% Saturation	100% Saturation
	PPM	0 PPM	5000 PPM
	Deg	-180	180
O_2 Medium Level	PPB Dissolved	0 PPB	5000 PPB
	PPM Gas Phase	0 PPM	5000 PPM
	Deg	-180	180
O_2 Low Level	PPB Dissolved	0 PPB	5000 PPB
	PPM Gas Phase	0 PPM	5000 PPM
	Deg	-180	180

pH	pH	Scale read for the channel from DSPCALIB.DAT in the USB-KEY (Factor 2 and Factor 3 for channels 2 and 4 in Figure 4-9)	
	SMR	0	3
CO_2	mmHg	0 mmHg	High scale value read for the channel from DSPCALIB.DAT in the USB-KEY (Factor 1 for channel 3 in Figure 4-9)
	%	0%	
	SMR	0	3

The output range of the analog output is as follows:

- Minimum measurement (4mA) is 2V output
- Maximum measurement (20mA) is 9.9V

5 Calibrating Sensors, General

Calibration gas and solution:

The DSP4000 typically only requires a 1-point calibration at a value near the range in which you will be measuring:

- pH measurements, use a buffer standard of 8.0.
- When a 95% O2/5% CO2 gas mix is the gas used for aeration and pH maintenance of your perfusion buffer, this gas can be used for the 1 point calibrations of both Oxygen and Carbon Dioxide (gas-saturate a non-bicarbonate buffered solution). If your perfusion system requires buffers to be deoxygenated (such as in lung applications) or use buffer equilibrated with room air, then air-saturated solution (non-bicarbonate buffered) should be used for calibration. In these situations it is common to use some 5% CO2 gas mix for the 1 point calibration of the CO2 sensor (such as 5% CO2) with balance air, or 95% N2/5% CO2.

***TECHNICAL NOTE*: Oxygen Sensor calibration is always performed based on air saturation and not dissolved oxygen saturation. 20.9% DO = 100% Air Saturation. 95% DO = 454% Air Saturation**

If a 2 point calibration needs to be performed, then the following zero/low standards should be used:

pH: buffer pH 5 or lower (this is the minimum output of the Polestar)

CO2: Room Air-equilibrated distilled water, which has a CO2 level of 0% or Nitrogen Gas

O2: pO2 zero solution (Catalog Number 73-3812) or Nitrogen gas saturated solution

The optical sensing films consist of fluorescent indicator chemistries immobilized in either hydrophilic (pH sensor) or hydrophobic (DO and CO2 sensors) polymer membranes. The gaseous (O2/CO2) or ionic (H+) species of interest move freely between the solution and interior portions of the sensing membranes to maintain an equilibrium of partial pressure (O2, CO2) or chemical potential (pH). The time to reestablish the equilibrium condition following a change in the

solution is dependent upon the species rate of diffusion through the membrane (faster at 37°C). For the purpose of calibration, it is recommended that the user allow a minimum of 10 minutes exposure of cycling the calibration solution through the sensor to ensure complete equilibration of the sensing film. Note: it is important that the calibration solution flow over the sensor during the calibration process. Failure to use a flowing calibration solution can affect the calibration results (flow through a flow-through sensor or use a magnetic stirrer).

If calibrating with gas, the gas should flow through the sensor for at least 5 minutes.

Application Note: If you wish to scale the OUTPUT of the DSP4000 for higher resolution within a smaller range of the total range of the sensor, use the DSP Configuration Utility (see section 4.6 "Scaling Values in the Calibration File" of the DSP4000 Optical Process Monitor User's Manual).

Temperature Influence on CO2 and O2 Measurements:

For accurate calibrations and measurements, the temperature needs to be known and actively compensated. You should have the temperature either manually set at the temperature at which the calibration is taking place (you can put a small temperature probe into the line to measure) or you can use an RTD probe that is wired into the DSP4000 unit. The RTD probe is the preferred method as you will have continuous temperature compensation and therefore will not need to change any temperature settings on the DSP4000 between calibration and measurement.

The DSP4000 can be configured such that a single RTD probe can provide the continuous temperature source for all channels. This is appropriate for a sensor like the Tri-fold Flow Cell. If sensors are located in different areas, then multiple RTD probes can be used. A Channel is instructed to look at either a Common Source RTD (for example, you can designate the RTD Probe on Channel 1 to be the Common Source and the other temperature-dependent sensor on the Tri-Fold Flow cell can be directed to use the Common Source). If another individual sensor is located elsewhere, then a second RTD probe can be installed on that channel and you would then direct the DSP4000 to use "RTD" as the source for that Channel.

If an RTD probe is not used, then the temperature can be set manually.

Manual Temperature Input

Manual Input allows you to specify the temperature value to use for compensation. This option should only be considered for applications where there is little or no variation in temperature or where the temperature is well regulated.

To set the temperature manually:

1. Select **Manual Input** in the Temperature Source menu and press ENTER to show the currently set temperature for manual input. The default is 20° C. The range is -20° C to 100° C.

2. Press the up or down arrow to increase or decrease the setting by 0.1° C, or hold the key down to rapidly change the value. Press the left or right arrow keys to change the position of the cursor to the tens, ones, or tenths position.

3. Press ENTER to accept the displayed setting and return to the Setup menu.

5.1 Set Barometric Pressure

Measurements taken for CO_2 and O_2 are sensitive to barometric pressure. Unless a pressure monitor is available with a 4-20mA analog output (which can be fed into the DSP unit), an accurate barometric pressure should be read from a NIST certified barometer and manually entered into the unit on a daily basis. Instructions for manual input of pressure are found in section 5.6.1 of the DSP4000 Optical Process Monitor User's Manual.

Options for **continuous barometric pressure compensation** are:

RM Young 61302L Barometer with 4-20mA analog output standard

Vaisala BAROCAP® Digital Barometer PTB330

Manual Pressure Input

You can manually enter a pressure value in units of mmHg or PSI. Input values must be absolute pressure, that is, barometric pressure plus gauge pressure. The default value is 14.7 PSI (760 mmHg). To use a manually input value for pressure compensation:

1. Select Pressure from the Setup menu and press ENTER to display the current setting, and then press ENTER to show the pressure input options.

2. Select one of the manual input options and press ENTER to show the currently specified input.

3. Press the up or down arrow to increase or decrease the setting one unit at a time, or hold down the key to rapidly change the value.

4. Press ENTER to accept the displayed value and return to the Setup menu.

5.2 Units Conversion, Data Acquisition 2-Point Calibration

After the DSP4000 Optical Process Monitor has undergone the 1-point calibration(s) for the channel(s) installed, you may proceed with applying the units conversion/calibration to your data acquisition system software.

For units conversion in LabChart, follow these steps:

1. Connect the Analog Output Cables to the Powerlab
2. Ensure that the Range is set at 10V on the Input Amplifier. Set up the channel appropriately in the Channel Settings Window or individually on each channel as per LabChart instructions
3. Press Start and record the upper calibration value that was used for the 1-point calibration. Once the reading is stable and you have your sample captured, proceed to step 4. Make note of the reading on the DSP4000 unit during this trace recording as this will be the value used in your units conversion.
4. Apply the low/zero conditions to the flowcell. For example, for oxygen this may be the upper calibration point of 95% O2 followed by buffer that has been aerated for 30 minutes or which is equilibrated with the aeration gas. Continue recording until the stable phase is reached and you have a suitable trace for calibration. Make note of the reading on the DSP4000 unit during this trace recording as this will be the value used in your units conversion.
5. Highlight the full calibration data trace (high and low point) for one sensor type at a time and choose "Units Conversion" from the right-click drop down for that channel. Follow normal calibration procedure using the values obtained from the DSP for the high and low points.

5.3 Oxygen Sensor Calibration

It is recommended to use the 1-point calibration on the DSP4000. When complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to the lower oxygen solution (such as aerated buffer), to capture the low value. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose "Units Conversion" from the drop-down menu. Use the values displayed on the DSP4000 for the 2 point calibration in LabChart.

5.4 Carbon Dioxide Sensor Calibration

A 1-point calibration should be performed on the DSP4000 when a known gas concentration, e.g. 5%, is not read accurately on the DSP Display. Typically this calibration point is at 5% as that is the concentration of CO2 that is used to maintain proper pH in most bicarbonate buffers used in perfusion systems. When the 1-point calibration is complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to the zero gas or solution for 5 or 10 minutes (respectively) (or until the reading has fully stabilized) and continue to collect data for the low point. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose "Units Conversion" from the drop-down menu and use the values that are displayed on the DSP4000 for the 2 point calibration in LabChart.

5.5 pH Sensor Calibration

When the pH reading on the DSP4000 is not accurate, a 1-point calibration can be performed on the DSP4000. A calibration buffer of a value near the pKa of the sensor is used. For a 5.5-10 pH sensor, the pKa, or midpoint of the response curve falls, at 8.2, therefore we choose pH 8 as the preferred calibration buffer for this sensor. When complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to pH 6 or other lower pH solution that falls below your optimal area of interest. Calibration Solution for 10 minutes (or until the reading has fully stabilized) and continue to collect data. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose "Units Conversion" from the drop-down menu and use the values that are displayed on the DSP4000 for the 2 point calibration in LabChart.

6 Viewing Data on DSP4000

Run Mode will by default show you the channel measurement data for the sensor plugged into that channel. The AUX button changes the display to give current readings of other channel-specific information:

If the AUX button is pressed, it scrolls the screen through displays of temperature/pressure, AGC Value, and Channel Measurements.

6.1 Channel Readings – O2, CO2, pH Parameters

These are displayed as in the image to the left. The channel designation followed by the numerical value and then the units that have been assigned to that channel.

This display is showing a three channel DSP4000 with two channels disabled.

6.2 Channel Readings – Temperature and Pressure Readings

Pressing the AUX key will give you a display that reads the channel designation followed by the temperature/pressure values per channel. These will only show real time data from an actively compensating input source (barometer or RTD probe).

6.3 Channel Readings – AGC Readings

These readings can be useful for feedback regarding sensor effectiveness or placement in regards to the fiber optic cable. High AGC readings indicate a high gain applied to the LED which can be caused by photo-bleaching of the sensor chemistry or by improper alignment of the chemistry in relation to the fiber optic cable.

7 Changing expired sensors

The Status LED in the upper right corner of the DSP4000 front panel provides a visual indication of the AGC or RAGC level during operation. The Status LED is:

- **Green** when the AGC level is less than 3000 for all four channels
- **Yellow** when the level for least one channel is between 3000 and 3500
- **Red** when the AGC for one channel exceeds 3500. An AGC or RAGC reading of 3500 or higher indicates a need to replace the sensing element, or an obstruction or break in the optical path.

When the Status light is **red**:

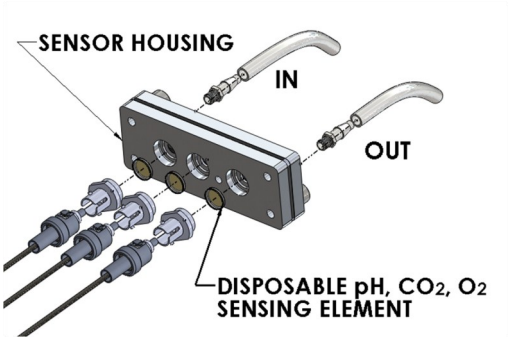
1. Check the AGC level of each channel to identify the channel causing the Status LED alert. See "AGC Display" Section 5.8 of the DSP4000 Optical Process Monitor User's Manual for information on accessing the display from the Setup menu.
2. Inspect the BioProbe connections and the optics on the problem channel to determine whether the high AGC is caused by an obstruction or break in the optic path. See "Inspection Procedures" in Section 6.1.1 of the DSP4000 Optical Process Monitor User's Manual.
3. Replace the sensing element if the AGC is still high after resolving any problems with the fiber optic path.

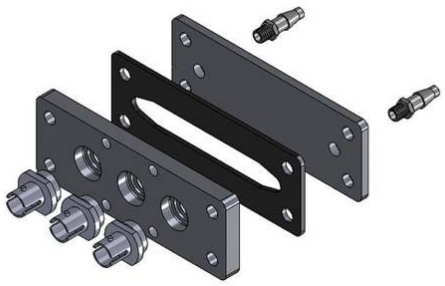
Flow-cells (glass insert with chemistry painted internally):

1. Remove the glass insert by loosening the screw with an allen/hex wrench and sliding the flow cell out.
2. Contact Harvard Apparatus or your Harvard Apparatus Appointed Distributor for the correct re placement part number for the flow cell insert for your measurement (i.e. O2 PPB, O2 PPM, O2 Sub-PPB, CO2, pH 5.5 – 10 or 4 - 7).

Tri-Fold sensor:

1. Remove the Fiber Optic cable(s).
2. Using a wrench (if unable to remove with fingers), unscrew the ST Connector on the channel that needs a replacement sensing disk.
3. Contact Harvard Apparatus or your Harvard Apparatus Appointed Distributor for the cor- rect replacement part number for your sensor type (*i.e.* O_2 PPB, O_2 PPM, O_2 Sub-PPB, CO_2, pH 5.5 – 10 or 4 - 7).





Puncture probe:

1. Remove the puncture probe from the fiber optic cable. This sensor is purchased as in the catalog as it has no replacement portions as it is a disposable item.
2. Update the configuration file on the USB-KEY, as described in "Loading the File onto the USB-KEY" Section 3.4 of the DSP4000 Optical Process Monitor User's Manual.

BioProbe:

1. Unscrew the tip from the BioProbe by twisting it counter-clockwise.

CAUTION: The sensing element is designed to seal when finger-tight. Never use pliers or any other tool to loosen or tighten the sensing element, as the tools can damage the sensing element and probe body.

2. Screw the replacement element onto the end of the probe. Calibration information for the sensing element is contained in a calibration file that includes the sensor type and the calibration factors for all elements in the same manufacturing lot. You can download the calibration file from the Support area on the Polestar Technologies web site.
3. Update the configuration file on the USB-KEY, as described in "Loading the File onto the USB-KEY" Section 3.4 of the DSP4000 Optical Process Monitor User's Manual.

7.1 Updating Lot Number for New Sensor Calibration

See Chapter 3 ("Managing Configuration Files) of the DSP4000 Optical Process Monitor User's Manual for using the Configuration Utility to assign new lot numbers to the measurement channel. The configuration utility is also used to reassign channels to different measurements.

8 Cleaning and Maintenance

Optical Sensors are not affected by ambient light when in use; however, they are sensitive to light and should be stored in the dark when not in use.

The sensors can be cleaned using MUCASOL, which is preferred when using the sensors in conjunction with a Hugo-Sachs Isolated Organ or Tissue Perfusion System whereby there are wetted components constructed from Plexiglas.

If the fiber optic cables have been detached and stored or if they are otherwise possible dirty you should use a piece of scotch tape to tap on the tip to remove particulate matter.

Additional in-depth maintenance information can be found in Chapter 6 "Care and Maintenance" of the DSP4000 Optical Process Monitor User's Manual.

You will need the following items to inspect and maintain the DSP4000:

- De-ionized water
- Chem Wipe® or soft lint-free cloth
- Scotch tape
- Magnifying glass
- Compressed air or nitrogen
- Adjustable wrench.

All Polestar sensors (including sensing elements, iDots, peel and sticks, and puncture probes) are sensitive to ambient light and will photo-bleach over time unless stored in a dark place between uses. Bright sun light will accelerate the aging process of the sensor. Photo-bleaching affects the useful life of the sensing element, but not the accuracy of measurements.

Troubleshooting Guide

Symptom	Explanation of Problem	Action
The DSP4000 does not display measurements or menus.	Insufficient or too much power is being applied to the DSP4000.	1. Disconnect power from the DSP4000. 2. Verify that the voltage is 24 VDC and the current is at least 1.5 Amps. 3. Re-connect the power.
The DSP4000 powers up with a USB error.	The USB-KEY is not recognized.	1. Re-seat the USB-KEY. 2. Press **ENTER**.
The DSP4000 does not go into Run mode, or kicks out shortly after startup. The Status LED is red.	The AGC for at least one channel has reached 4000, the maximum value because: • There is a poor optical connection. • A sensor element is at the end of its life.	1. Verify all optical connections. 2. Check the AGC for each channel. 3. Replace sensing elements as needed.
The Status LED is yellow.	The AGC for at least one channel has reached 3000 because: • Dirt has accumulated on an the optical surface. • The sensing element is near the end of its useful life.	1. Check the AGC for each channel. 2. Clean the optical surfaces using Scotch tape as dust and dirt particles may result in signal loss. 3. Replace sensing elements as needed.

If none of the above apply and you are seeing "AGC Limit," please check to make sure that the sensor chemistry is as close to the LED/fiber optic as possible. If using a glass flow-cell, check to make sure that it is seated in the cell mount such that the chemistry is between the light and the perfusate/media.

Ordering Information

31165	O_2 Replacement Kit (sensor, glass window & O-Ring)
31166	pH Replacement Kit (sensor, glass window & O-Ring)
31167	CO_2 Replacement Kit (sensor, glass window & O-Ring)
31168	O_2 Replacement sensor
31175	BUFFER SOLUTION KIT: pH 6, 7.41, 8, 500ml/EA
31176	Trifold Connections Kit 1/16" with sensors

Appendix B: PID Controller Theory & Application

This section is intended to provide additional information about the theory and application of a PID control loop.

PID control uses a feedback mechanism to minimize disturbances and keep the system close to the user defined set point at any given time. The set point can be a constant, a linear function, or even a sinusoidal function.

PID control is named after its three correcting terms (proportional, integral, and derivative). The weighted sum of these three parameters calculate the output of the PID controller. Put simply, P depends on present error, I on the collection of past errors, and D is a prediction of future error.

The ORCA uses PID control to regulate pressure. After measuring the pressure and calculating the error (error = set point - actual value), the controller decides when to change the flow rate and by how much. For example, if the current pressure reading is 60mmHg, and the desired set point is 80mmHg, the controller will increase the flow rate in order to make up the difference. If the set point is instead 120mmHg, the controller will increase the flow rate more rapidly. This is known as proportional control (P). If the desired pressure is not being reached quickly enough, the controller may try to speed up the process by running the pump faster and faster as time goes by; this is considered integral control (I). However, making changes that are too large for a small amount of error leads to overshoot. Repeated changes that are too large leads to an output that oscillates around the set point. Derivative control (D) can be used in order to dampen these oscillations by making inferences about the future based on the slope of the error function at a given point.

The user has the ability to set values for these parameters. Generally, the proportional term (P) should constitute the majority of the output change. A small P value results in a small output response to a large input error. In other words, the controller will take a long time to offset the error, but generally the system will be stable. In contrast, a large P value will offset any errors quickly, but at the risk of system instability.

The integral term is useful for accelerating the process towards set point and for eliminating residual steady-state error that could occur using a pure proportional controller. However, it can cause the present value to overshoot the set point value.

The derivative term is currently not used with the ORCA due to its inherent sensitivity to measurement noise. A large, sudden change in measured pressure could cause erratic changes in the control mechanism that degrade performance.

In physiological systems, it is generally more important to minimize overshoot than to have a very fast response time with respect to error. Run the system and track data for about 5 minutes. If the system seems too unresponsive, the correct course of action would be to slowly increase the P value. If there seems to be too much overshoot, the I value should be lowered. There is no easy answer to finding the optimal values for these parameters; it is simply a matter of adjusting them based on observations and testing. When in doubt, typically conservative (i.e. lower) values for I and P are preferred.

Commonly used terms in PID systems:

Set Point - the desired value for a variable at a point in time

Process Variable - the actual (measured) value of that variable

Error - the difference between the set point and the process variable at a given time

Tuning - optimizing each of the three parameters such that rise time, overshoot, settling time, and steady state error are minimized.

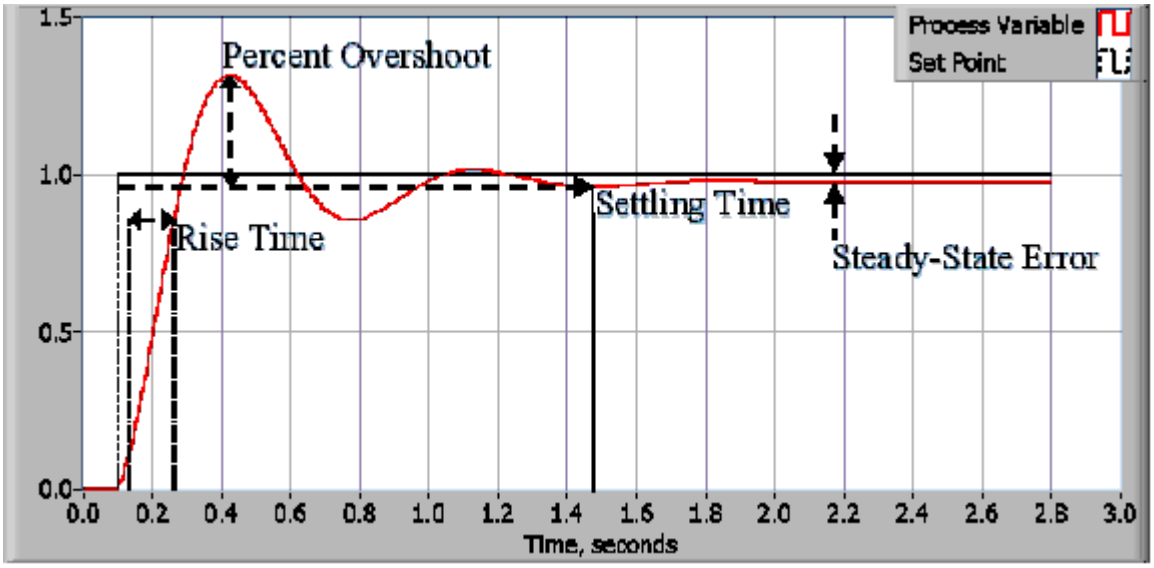
Steady-State - the final value that the system achieves in which an equilibrium is maintained

Steady-State Error - the final difference between the process variable and set point (ideally zero)

Rise Time - the time that is takes the system to go from 10% to 90% of the steady-state value

Overshoot - the amount that the process variable goes above the final value

Settling Time - the time required for the process variable to settle within 5% of the final value



The following table may be of use when tuning the PID controller:

Effect of increasing a parameter independently

Parameter	Rise Time	Overshoot	Settling Time	Steady-State Error	Stability
K_p	Decrease	Increase	Small change	Decrease	Degrade
K_i	Decrease	Increase	Increase	Eliminate	Degrade
K_d	Minor change	Decrease	Decrease	No effect (in theory)	Improve (if K_d small)

Bioreactor PID Temperature Controller

A HART PID Controller with blanket heater can be ordered as an accessory in order to regulate temperature in either the reservoir and/or (pictured below) in the chamber.

Setup Instructions

Select the appropriate power cord and insert into the power cord plug.

NOTE THE REVISION 1 PID CONTROLLERS ARE 100V ONLY

The unit should ONLY be plugged into a 220V-100V transformer if the line voltage is 220V

Do not connect temperature probe until you have finished setting thermocouple type and Set Point temperature.

Select the appropriate ORCA temperature probe and plug the connector into the port using the color coding.

All four types of temperature probes will work with this unit

12″ (30.5cm) L x $^{1}/_{8}$″ (.32cm) OD
12″ (30.5cm) L x $^{1}/_{16}$″ (.16cm) OD
6″ (15.25cm) L x $^{1}/_{8}$″ (.32cm) OD
6″ (15.25cm) L x $^{1}/_{16}$″ (.32cm) OD

Programming Instructions

Setting Thermocouple Type

1. Press SET to enter password menu. Bottom display will show 0000.	
2. Enter 0089 using arrow buttons. a. Use > button to change selected digit, down/ up buttons to change digit value. b. Press SET to enter 0089 menu.	
3. IntY is the first item in 0089 menu. Press SET to edit IntY, which is the thermocouple type setting. a. Default value is P100, use down/up buttons to scroll to t. The Bioreactor PID Temperature Controller is only compatible with type-T thermocouples. b. Press SET to save thermocouple type. You will be returned to 0089 menu.	
4. No other settings in the 0089 menu need to be changed. Use the down/up buttons to select END, and press SET to return to main screen .	

Inputting Temperature Set Point Value

Note: If the user allows the PID controller to idle for about one minute, the controller will return to the main menu. If this happens, simply follow the above steps again to get back to the desired menu.

1. Press SET to enter password menu. Bottom display will show 0000.	
2. Enter 0001 using arrow buttons. a. > button to change selected digit, down/up buttons to change digit value. b. Press SET to enter 0001 menu.	
3. SV is the first item in the 0001 menu. Press SET to edit SV, which is the Set Value. a. Default value is 0800, use arrow buttons to change to desired value. Example photo is set to 0040. b. Press SET to save SV temperature. You will be returned to 0001 menu.	
4. No other settings in the 0001 menu need to be changed. Use the down/up buttons to select END, and press SET to return to main screen.	

1. Verify device is now at main screen, showing thermocouple temperature in top red display, and desired Set Value in lower green display.	
2. Press and hold > button until AT light blinks. OUT light will also be flashing, at different intervals, showing heater output status.	
3. Auto Tuning should take several minutes, depending on chamber size to be heated. **When tuning is complete, AT light will stop blinking and remain off.** The PID Controller has now determined the correct heating parameters. OUT light will continue to flash as the PID controller maintains the Set Value. The PID Controller will save the settings after powering off, so Auto Tuning should not need to be run more than once for a given configuration. If desired, Auto Tuning can be run again by pressing and holding the > button.	

Note: The first time running the PID controller, it is recommended that warm water is used to reduce the amount of overshoot as much as possible. The PID control will test limits and will intentionally overshoot and undershoot the set point range which can damage an organ. After the auto tune function has been used once, it is ready to use with an organ.

Appendix C: Frequently Asked Questions & Troubleshooting

ORCA Bioreactor

Chambers

What are the sizes of the chambers available?

There are three different sizes of chambers available: 14", 10", and 5.5" in diameter. The right selection is simply based off of the size of the organ being studied.

How are the chambers sterilizable?

Chambers and their components are designed to be sterilized by normal laboratory methods such as autoclaving, EtO and plasma sterilization.

How are the chamber lids designed?

Multiple ports are available in the top lid of the chamber to accommodate the various cannulation and access requirements.

For what range of pressures are the chambers designed?

The chambers are designed to hold physiological pressures. They are NOT designed to be used as pressure chambers which allow 1atm/15psi to be applied. The chambers can handle negative ventilation at several psi.

Pumps

What are the maximum/minimum flow rates achievable by the peristaltic pumps?

The peristaltic pump drive units are very flexible in their design and can have up to four heads on each drive unit. Each of the heads can be either single or dual channel. The single channel heads allow a flow rate of 1.2L/min. Therefore, the maximum rate per pump drive unit is 4.8L/min (by using 4 pump heads). This is sufficient for the majority of applications. In the case of large animal (porcine or human) hearts, an average of approximately 5L/min must be achieved generally. This means that typically 10-11L/min is needed during systole, requiring a large pulsatile pump. This pump operates by filling a chamber and a piston drives it in one pulse to the system.

The flow rates are constrained by the type of tubing that is used. The smallest tubing (1/32" ID) allows flow rates from 0.018mL/min, and the 3/8" ID tubing allows up to 4.8L/min per pump. The pulsatile pump is often equipped with 1/2" ID tubing.

Controller

How many channels can the controller support?

The ORCA 4 controller allows the use of four peristaltic pumps allowing maximal flexibility to support even the most demanding protocols. Typically, three pumps are used and the fourth is available for specialty configurations. Three pumps may be initially purchased and the user may add the fourth pump on later. For applications involving large animal organ perfusion, a pulsatile pump is typically used in order to achieve the necessary volume.

Can the software be installed on the computer of my choosing?

The ORCA software is typically run from the laptop provided. It can be run from a standard desktop PC as long as it is configured properly. If the user wishes to use a desktop, HART may configure it in our laboratory. If an on-site installation is planned, it may be able to be configured then.

Heater/Temperature Regulation

How is temperature regulation achieved?

The reservoir heater is a major source of heat and temperature maintenance for the system but it not sufficient as a standalone. All chambers use at least one wraparound heater in conjunction with a PID system in order to maintain physiological temperatures.

It is recommended that the user preheats the medium in an incubator to the required temperature before introducing it to the reservoir. The reservoir can be fed from either a bag or a large feed bottle by using one of the dual channel heads of the peristaltic pumps. The slow introduction of medium reduces the chance of contamination since the circuit is rarely opened.

The reservoir heater is limited to a maximum temperature of 39.5^{o} C so that the medium is not denatured. If medium from a cold room (4^{o} C) is introduced, the time required to raise the temperature to 37^{o} C will be very lengthy.

The reservoir bottles come in various sizes and with different ports to accommodate a wide variety of protocols.

How is temperature monitored?

The ORCA controller provides the ability to use four temperature probes. A temperature probe is normally placed in the reservoir to control heating functions and heat the medium when the temperature dips below the set point (with a maximum of 39.5^{o} C). Another probe is used to monitor the temperature within the chamber itself. An implantable temperature probe can also be used to measure temperature inside an organ. Additionally, users may wish to add a second reservoir to the system and monitor its temperature.

What are the types of temperature probes available?

There are 6" and 12" permanent temperature probes available, as well as a flexible implantable probe.

Gas Monitoring System

How are gas monitoring (CO_2, O_2) and pH monitoring typically performed?

The system that HART currently uses is purchased from Polestar and performs with a high degree of precision and accuracy. The system provides the ability to monitor CO2 and pH from a single point (typically the reservoir). Another ability is to measure the pre– and post-organ oxygen levels, allowing for easy determination of the oxygen consumption rate of the organ.

Oxygenating System

How are gas levels (CO_2, O_2) controlled?

The typical manner to oxygenate a system is to have media from the reservoir sent through the intraluminal side of an oxygenator using one of the channels of a peristaltic pump.

There are a wide variety of oxygenators. The D150 is used for small animal organs as it minimizes the volume and has shown the capacity needed for rodents to rabbits. For large animals, the higher capacity D200 is often used. In the past, we have seen clinical oxygenators such as Maquet, Terumo and Medtronic also used. The life span of the oxygenators are dependent on the medium and additives used as well as individual protocols.

The extraluminal space of the oxygenator is typically supplied with 95% air / 5% CO_2. This can be done with a premixed tank of gasses or a gas mixer. The OKO system allows the user to feed in CO_2 from a tank and air. The air can be supplied from a tank, from an air compressor, or house air. If house air is used, it is recommended that a moisture trap be added.

Pressure Transducers

How many pressure transducers can be used with the system? What are their capabilities?

The chamber is built to allow for up to four pressure transducers per ORCA system. There are multiple types of pressure transducers that can be used with the system. Typically single-use Pendotek pressure transducers are utilized. These are not autoclavable although some users have reused them following EtO or chemical sterilization. It is currently unclear how long the units last after being cleaned and sterilized. The peristaltic pumps can be used in a constant pressure mode or pressurized for negative ventilation.

Troubleshooting

ORCA Software

Problem: An error message is displayed when using the ORCA software.

- From the Notifications menu, click "Status", then uncheck the "Enable" box. Next click "Save" and use the check mark to exit the screen.

Problem: The ORCA software immediately closes upon start-up.

- Check the following:
  - Windows auto-updates is turned off, otherwise it could cause the computer to restart whilst running an experiment.
  - The ORCA power cord is plugged into the ORCA controller and the wall
  - The Communications cable is plugged into computer and into the ORCA controller
  - The ORCA controller is turned ON
  - Your computer's static IP should be set to 172.16.51.59 (the subnet mask should be 255.255.255.0). DO NOT PLUG THE COMMUNICATIONS CABLE INTO A ETHERNET PORT AND CONNECT IT TO THE UNIVERSITY/INSTITUTION NETWORK. THE LAPTOP COULD CHANGE THE IP ADDRESS AND NOT BE ABLE TO COMMUNICATE CORRECTLY WITH ORCA. YOU WILL THEN HAVE TO MANUALLY CHANGE THE IP ADDRESS BACK TO 172.16.51.59 (the subnet mask should be 255.255.255.0).

Peristaltic Pumps

Problem: There is an issue with the peristaltic pumps concerning the backfill siphon of the reservoir into the chamber.

- Watch how the pump rollers stop to ensure that they always occlude the tubing.

UPDATE LOG

Date	Revision	Changes Made
13-Oct-01	1.3.1	Created update log. Added thermocouple ordering information (pg15).
13-Oct-08	1.3.2	Created new example config schematic for 5.5” chamber lung system (pg29). Fixed minor error in PharMed tubing specifications chart (pg41).
13-Oct-22	1.4.1	Updated part numbers and drawings. Added in Blanket Heater instructions. Updated FAQs and troubleshooting section.

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